Metabolism of the Surfactants Sodium Undecyltriethoxy Sulphate and Sodium Dodecyltriethoxy Sulphate in the Rat

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The metabolic fates of the synthetic surfactants, sodium [1-¹⁴C]undecyltriethoxy sulphate and sodium [1-¹⁴C]dodecyltriethoxy sulphate were studied in the rat. Both compounds were extensively metabolized regardless of the route of administration, oral, intra-peritoneal or intravenous. Short-chain radioactive products were eliminated in the urine: the major metabolite of the dodecyl homologue in the urine was identified as ⁻O₂C¹⁴CH₂-(OC₂H₄)₃OSO₃⁻ by n.m.r. and g.l.c.-mass spectrometry, whereas the major metabolite of the undecyl homologue in the urine was tentatively identified as ⁻O₂CCH₂¹⁴CH₂-(OC₂H₄)₃OSO₃⁻. In contrast with experiments with the dodecyl derivative, when [1-¹⁴C]undecyltriethoxy sulphate was administered to rats, appreciable amounts of radioactivity were recovered as ¹⁴CO₂ in expired air. Whole-body radioautography implicated the liver as the major site of metabolism of both surfactants. The nature of the metabolic products establishes that both compounds are degraded by ω,β -oxidation. Cleavage of the ether linkage proximal to the sulphate moiety may account for the small amounts of ¹⁴CO₂ recovered in expired air after the administration of [1-¹⁴C]dodecyltriethoxy sulphate. It is suggested the substantial amounts of ¹⁴CO₂ recovered after [1-¹⁴C]undecyltriethoxy sulphate administration originate from ⁻O₂¹⁴C(OC₂H₄)₃OSO₃⁻, an unstable product of ω,β -oxidation. An n.m.r. spectrum of the metabolite identified as 2-(triethoxy sulphate)acetic acid and a mass spectrum of the trimethylsilyl derivative of the parent alcohol of that metabolite have been deposited as Supplementary Publication SUP50086 (5 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1978) **169**, 5.

Alkylethoxy sulphates, R-(OCH₂CH₂)_n-OSO₃Na, are used as anionic surfactants in household detergent products of many varied uses (Schwartz *et al.*, 1958). As a result of the widespread usage of these surface-active agents, a number of toxicity studies have been carried out (e.g. with dodecyltriethoxy sulphate; Walker *et al.*, 1967) that suggest that alkyltriethoxy sulphates have a low toxicity. The biodegradability of this class of surfactant in sewage and industrial waste by micro-organisms has received some attention (for a review, see Swisher, 1970), but metabolic studies and identification of degradation products have not been reported. Further, no detailed study of the metabolic fate of these surfactants appears to have been carried out in mammals. Studies on the fate *in vivo* of the closely related alkyl sulphates and the alkyl sulphonates in various mammals have shown that the major route of degradation for all the straight-chain surfactants studied is by way of ω -oxidation of the terminal methyl group followed

by β -oxidation of the alkyl chain of the resultant fatty acid derivatives. Thus dodecyl sulphate is degraded in rats (Denner *et al.*, 1969) and is also degraded in fish (Tovell *et al.*, 1975); decyl and octadecyl sulphates are degraded in rats (Burke *et al.*, 1975) and hexadecyl sulphate is degraded in man, dogs and rats (Merits, 1975). The major end-product of degradation of all these anionic detergents with even-numbered carbon chains is butyric acid 4-sulphate, which appears in urine, although some non-enzymic desulphation of the butyric acid 4-sulphate formed takes place *in vivo* (Ottery *et al.*, 1970). In man and dogs some glycolic acid 2-sulphate is also formed (Merits, 1975) indicating that in these mammals steric hindrance by the sulphate moiety does not preclude removal of another two-carbon fragment from butyric acid 4-sulphate. The odd-numbered-carbon-chain surfactant undecyl sulphate is similarly biodegraded in the rat by ω,β -oxidation to short-chain carboxylic acid derivatives which are eliminated in the urine (Burke *et al.*, 1976). The surfactants, dodecyl and hexadecyl sulphonates, are metabolized in a similar fashion to butyric acid 4-

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sulphonate as the sole end-product of biodegradation (Taylor *et al.*, 1976). This metabolite is eliminated in the urine and thus both the alkyl sulphate and alkyl sulphonate surfactants are biodegraded *in vivo* by ω,β -oxidation.

The two compounds selected for the present study are the sodium salts of undecyltriethoxy sulphate and dodecyltriethoxy sulphate. Both have triethoxy sulphate groups in common but differ in the length of the alkyl chain. On the basis of previous studies in these departments different metabolic end products would be expected. Further additional sites for metabolic degradation are provided by the ether links in the ethoxy groups, which may be susceptible to attack by an enzyme system described by Tietz *et al.* (1964). The identity of the breakdown products *in vivo* was investigated in order to determine the mechanism(s) of degradation of alkyltriethoxy sulphates in the rat. The metabolism of undecyl and dodecyltriethoxy sulphates was investigated in order to ascertain the effect of varying the length and nature of the alkyl chain on the rate of absorption, extent of metabolism and the route and rate of excretion of alkyltriethoxy sulphate surfactants and their degradation products. A preliminary account of some of the material described in this paper has been presented (Taylor *et al.*, 1976).

Materials and Methods

Labelled surfactants

Sodium [1-¹⁴C]undecyltriethoxy sulphate and sodium [1-¹⁴C]dodecyltriethoxy sulphate were prepared by Mr. C. T. James (Unilever Research, Colworth/Welwyn Laboratory, Sharnbrook, Bedford MK44 1LQ, U.K.). The appropriate [1-¹⁴C]alkanol, prepared as described by Taylor *et al.* (1977), was converted to the tosyl derivative, condensed with monosodium triethyleneglycol to produce the required [1-¹⁴C]alkyltriethoxy alcohol and sulphated with chlorosulphonic acid. The radiochemical and chemical purities of the intermediate [1-¹⁴C]alkanols and [1-¹⁴C]alkyl triethoxy alcohols were checked by using t.l.c. in solvents A, B and C (*vide infra*), radio-g.l.c. and mass spectrometry. These techniques confirmed the structures and showed each sample to be >97% pure.

The purity of the alcohol triethoxy sulphates was determined by t.l.c. in solvents A, B and C (*vide infra*), radio-g.l.c. and reverse isotope dilution analysis. Both sodium [1-¹⁴C]undecyltriethoxy sulphate (specific radioactivity 4.16mCi/mmol) and sodium [1-¹⁴C]dodecyltriethoxy sulphate (specific radioactivity 3.04mCi/mmol) were found to be >97% radiochemically and chemically pure by these methods.

Characterization of sodium [1-¹⁴C]undecyltriethoxy sulphate and sodium [1-¹⁴C]dodecyltriethoxy sulphate

The i.r. spectra of samples of sodium [1-¹⁴C]-undecyltriethoxy sulphate and sodium [1-¹⁴C]-dodecyltriethoxy sulphate showed strong absorption bands at 2850cm⁻¹ due to -CH₂- groups and weaker absorption bands at 1470cm⁻¹ and 1380cm⁻¹ due to -CH₂- and -CH₃ groups. The sulphate group is characterized by the appearance of strong absorption bands at 1040-1200cm⁻¹, and the presence of absorption bands at 1100cm⁻¹ and 1250cm⁻¹ are indicative of the presence of ether linkages.

On hydrolysis in 0.1M-HCl at 100°C for 3h, a sample of sodium [1-¹⁴C]undecyltriethoxy sulphate gave a single peak on radio-g.l.c. at 200°C with a relative retention time (1.44) intermediate between that of authentic decyltriethoxy alcohol (1.00) and dodecyltriethoxy alcohol (2.10). This confirmed that the alkyl chain contained 11 carbon atoms.

A portion of sodium [1-¹⁴C]dodecyltriethoxy sulphate was analysed by field desorption mass spectrometry using methanol/water (1:1, v/v) as solvent and a wire current of 15mA. The resultant mass spectrum showed predominant ions at *m/e* 863 and 443 which represent (2*M*+Na)⁺ and (*M*+Na)⁺ ions respectively. Indications of a molecular ion at *m/e* 420 and the presence of ions at *m/e* 169 and 251, produced by fragmentation of the molecule at the ether link adjacent to the alkyl chain to produce (C₁₂H₂₅)⁺ (*m/e* 169) and [(OC₂H₄)₃SO₃Na]⁺ (*m/e* 251), confirm the structure of the surfactant.

Storage

The sodium [1-¹⁴C]alkyltriethoxy sulphates were stored as solids, desiccated at -20°C, and dissolved as required immediately before use. The purity of the [1-¹⁴C]alkyltriethoxy sulphates did not change over the period of study (approx. 4 months) as determined by t.l.c. and radio-g.l.c. of the hydrolysate.

Infrared spectroscopy

Samples were prepared as KBr discs and spectra were obtained on a Perkin-Elmer 257 Grating Infra-red spectrometer.

Nuclear-magnetic-resonance spectroscopy

The spectrum of the major urinary metabolite from sodium [1-¹⁴C]dodecyltriethoxy sulphate was obtained on a Varian XL-100MHz machine fitted with a Fourier transform capability by Mr. A. G. Flook (Unilever Research), who also interpreted the spectrum.

Mass spectrometry

The synthesized detergents and the trimethylsilyl derivatives of metabolites from sodium [1-¹⁴C]-dodecyltriethoxy sulphate were analysed by Dr. D. E. Games (Department of Chemistry, University

College, Cardiff, U.K.) on a Varian CH5 double-focusing instrument. Samples of the surfactants were analysed using the technique of field desorption mass spectrometry (Games *et al.*, 1974).

Thin-layer chromatography

Chromatography was performed on silica-gel H plates in the following solvent systems: A, butan-1-ol/acetic acid/water (4:1:1, by vol.); B, chloroform/methanol/water (65:25:4, by vol.); C, propan-2-ol/water (7:3, v/v). Radioactive areas on chromatograms were located with X-ray film. For preparative studies radioactive zones on t.l.c. plates were located by radioautography and silica gel corresponding to the radioactive areas was collected and eluted with water.

Radio-gas-liquid chromatography

A Pye 104 gas chromatograph was linked to a Panax radiogas detector (Panax, Redhill, Surrey, U.K.). The glass column (1.5m×4mm internal diameter) was packed with either 3% (w/w) SE-30 on Chromosorb W 100–120 mesh (Hewlett-Packard, Wokingham, U.K.) or 3% (w/w) OV-17 on Chromosorb W 80–100 mesh (Supelco Inc., Bellefonte, PA, U.S.A.) and conditioned at 250°C before use.

Volatile derivatives of metabolites were chromatographed with a temperature programme of 180–250°C at 4°C/min for the trimethylsilyl derivative of the metabolites from [1-¹⁴C]dodecyltriethoxy sulphate and at 150–210°C at 4°C/min for the trimethylsilyl derivatives of [1-¹⁴C]undecyltriethoxy sulphate. The carrier gas (argon/CO₂, 19:1) flow-rate was 100ml/min, the flame ionization detectors were maintained at 275°C and received 50ml of hydrogen/min and 750ml of air/min.

Experimental animals

Male and female albino rats of the Colworth Wistar strain (approx. 200g body wt.) were used for preliminary metabolic studies. Compounds were administered under ether anaesthesia either orally or intraperitoneally to animals which were placed in separate metabolism cages that permitted the collection of urine, faeces and ¹⁴CO₂ in respired air. Food and water were available *ad libitum*. Urine samples were collected 6, 24 and 48 h after administration and a single collection of faeces was made at 48 h. The amount of ¹⁴CO₂ in respired air was measured hourly for the first 6 h and then at 24 h, by sampling the trapping fluid (aqueous ethanolamine, 50% by vol.).

M.R.C. hooded rats were used for experiments in which the surfactants were administered intravenously to wholly anaesthetized animals. Animals were anaesthetized with ether, the jugular vein was cannulated towards the heart and anaesthesia was maintained by the introduction of Nembutal (Abbot Laboratories, Queenborough, Kent, U.K.; 10–15

mg/kg body wt.) as necessary. The common bile duct and ureters were cannulated and hourly bile and urine samples were collected for 6 h after administration of the radioactive compounds via the jugular vein cannula. Respired air was collected by cannulating the trachea and connecting the cannula to an air reservoir (approx. 250 ml) linked in series with two gas wash-bottles containing aqueous ethanolamine (25%, v/v) and maintained under reduced pressure by a water pump. Samples of trapping fluid were removed hourly and assayed for radioactivity.

Whole-body radioautography

Young M.R.C. hooded rats (approx. 50g body wt.) received the compounds either orally or intraperitoneally (1 mg/animal) while under ether anaesthesia. Whole-body radioautograms were prepared by the method of Powell *et al.* (1967).

Detection of radioactivity

All samples were assayed for ¹⁴C in a Packard 2425 Tri-Carb liquid-scintillation spectrometer (Packard Instruments, Downers Grove, IL, U.S.A.). The counting efficiency of each sample was established by using an external-standard channels-ratio technique, which was calibrated with n-[¹⁴C]hexadecane as internal standard (The Radiochemical Centre, Amersham, Bucks., U.K.). All test solutions and biological fluids were assayed by counting the radioactivity of samples in PCS liquid scintillator (Amersham Searle, Chadwell Heath, Essex, U.K.). Faecal samples were freeze-dried, weighed, ground in a pestle and mortar and samples of up to 500 mg were combusted in an Inter technique IN4101 Oxymat sample oxidizer (Inter technique, 78370 Plaisir, France). Further samples of the freeze-dried faeces were extracted with methanol in a Soxhlet extraction apparatus. It was shown that the bulk (>99%) of the radioactivity was extracted after 3 h.

Experimental and Results

Oral administration

Male and female rats received either 10.25 μCi of sodium [1-¹⁴C]undecyltriethoxy sulphate or 7.24 μCi of sodium [1-¹⁴C]dodecyltriethoxy sulphate (0.2 ml of a 5 mg/ml aqueous solution) by gastric intubation. The radioactive contents of urine, faeces and respired air were determined and are recorded in Table 1.

In experiments with [1-¹⁴C]undecyltriethoxy sulphate the major route of excretion of radioactivity was urinary with relatively small amounts (up to 6.1%) associated with the faeces. However, considerable amounts of radioactivity were present in respired air (16.6–37.8%). The overall recovery of ¹⁴C was about the same in both sexes. However, there was a significant sex difference in the propor-

Table 1. *Recovery of ^{14}C in urine, faeces and respired air after oral administration of either sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate to male and female rats at a dose of 1 mg/animal*

For experimental details see the text. Results are mean values from the numbers of animals stated expressed as % of administered radioactivity, with ranges in parentheses.

		Recovery (% of administered radioactivity)			
		Sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate		Sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate	
	Time (h)	Males (3)	Females (3)	Males (4)	Females (4)
^{14}C content of urine samples	0-6	28.6 (3.5-42.0)	19.2 (18.7-19.6)	64.4 (51.1-76.8)	31.8 (11.8-39.8)
	6-24	37.5 (22.4-64.5)	24.3 (14.2-33.6)	28.3 (23.9-32.7)	46.8 (28.1-55.2)
	24-48	0.6 (0.3-0.7)	1.3 (0.7-1.6)	1.9 (1.3-2.2)	7.2 (4.1-10.3)
Total	0-48	66.7	44.8	94.6	85.8
^{14}C content of faeces	0-48	3.7 (2.5-4.3)	4.7 (2.1-6.1)	7.8 (4.0-14.8)	10.6 (6.0-12.4)
^{14}C content of respired air	0-24	19.4 (16.6-23.9)	35.2 (32.6-37.8)	1.1*	1.9*
Recovery (% of injected dose)		89.8	84.7	102.5	99.3

* Pooled determinations

tion of radioactivity excreted in urine and respired air. Thus 66.7% of the injected radioactivity appeared in the urine of male rats while the corresponding figure for females was 44.8%. The radioactivity recovered in expired air represented 19.4% in males and 35.2% in females.

In experiments with $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate only traces (up to 1.9%) of the radioactive dose appeared in respired air, some radioactivity (up to 14.8%) was associated with the faeces, but the vast majority (85.6% in females, 94.6% in males) was excreted in the urine.

The major differences in the results obtained with these two compounds were the relative amounts of radioactivity recovered in respired air, the extent of urinary excretion and the influence of sex on the route of excretion of radioactivity after the administration of the undecyl homologue.

Intraperitoneal administration

Male and female rats received, intraperitoneally, either 10.25 μCi of sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or 7.24 μCi of sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate at a dose of 0.2 ml of a 5 mg/ml aqueous solution and the results are recorded in Table 2.

With $[1-^{14}\text{C}]$ undecyltriethoxy sulphate the major excretory route of the radioactivity was urinary but some ^{14}C (up to 30.8% of the dose) was recovered in the expired air. In addition, the faeces of male rats receiving the surfactant contained up to 33.3% of the radioactive dose.

With $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate only small amounts of radioactivity (up to 3.2%) were present in respired air; substantial amounts (approx. 30%) were present in the faeces, but the major excretory route was urinary.

The major difference in the results obtained with the different surfactants was the relative proportions of the radioactive dose that appeared in the urine and expired air.

Intravenous administration

Wholly-anaesthetized male and female rats with bile duct and ureter cannulae received either 10.25 μCi of sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or 7.24 μCi of sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate intravenously at a dose of 1 mg/animal. Bile and urine samples were collected hourly for 6 h and the radioactive contents were determined. The results are shown in Table 3.

In experiments with $[1-^{14}\text{C}]$ undecyltriethoxy sulphate significant amounts (approx. 10% of the dose) of radioactivity were present in the bile of female rats, but in both sexes the majority of the excreted radioactivity was present in the urine. Some ^{14}C (up to 13%) was recovered in expired air.

In the corresponding experiments with $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate, the overall recovery of radioactivity was much lower (up to 51.6% of the dose) and the greatest proportion (approx. 95%) of the ^{14}C excreted was present in the urine.

Whole-body radioautography

Radioautograms were prepared from rats receiving either 10.25 μCi of sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or 7.24 μCi of sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate (at a dose of 1 mg/animal as in previous experiments) by the oral or intraperitoneal route and killed at time intervals ranging from 15 min to 24 h after administration. The radioautograms demonstrated that the liver and kidneys were the only organs in which cellular accumulation of radioactivity occurred, that is the concentration of ^{14}C

Table 2. *Recovery of ^{14}C in urine, faeces and respired air after intraperitoneal administration of either sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate to male and female rats at a dose of 1 mg/animal*

For experimental details see the text. Results are mean values expressed as % of administered radioactivity, with ranges in parentheses.

	Time (h)	Recovery (% of administered radioactivity)			
		Sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate		Sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate	
		Males (3)	Females (3)	Males (5)	Females (5)
^{14}C content of urine samples	0-6	30.4 (12.2-66.4)	30.1 (29.7-30.3)	40.3 (15.8-54.9)	50.9 (36.4-59.7)
	6-24	14.8 (4.1-24.7)	15.5 (6.5-26.4)	22.6 (8.2-45.6)	15.6 (7.9-25.7)
	24-48	1.2 (0.7-1.9)	1.3 (0.3-1.8)	1.0 (0.5-1.9)	1.1 (0.4-1.6)
Total	0-48	46.4	46.9	63.9	67.6
^{14}C content of faeces	0-48	22.8 (3.0-33.3)	6.0 (4.4-7.1)	33.7*	32.2*
^{14}C content of respired air	0-24	12.5 (11.0-15.0)	19.3 (10.1-30.8)	2.3 (1.8-2.7)	2.8 (2.6-3.2)
Recovery (% of injected dose)		81.7	72.2	99.9	102.6

* Pooled determinations

Table 3. *Recovery of ^{14}C in urine, bile and respired air after intravenous administration of either sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate to wholly anaesthetized male and female rats at a dose of 1 mg/animal*

For experimental details see the text. The results are individual values or mean values expressed as % of administered radioactivity with ranges in parentheses. Abbreviation: n.d., not determined.

	Time (h)	Recovery (% of administered radioactivity)			
		Sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate		Sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate	
		Males (2)	Females (2)	Males (3)	Females (2)
^{14}C content of urine samples	0-1	13.1 (11.6, 14.5)	7.9 (3.2, 12.6)	6.2 (2.9-7.1)	1.2 (1.1, 1.3)
	1-2	17.2 (14.8, 19.6)	15.1 (8.9, 21.3)	15.8 (11.5-18.9)	11.8 (9.9, 13.6)
	2-3	12.5 (9.3, 15.6)	17.4 (16.4, 18.4)	14.6 (10.7-18.5)	13.1 (11.6, 14.6)
	3-4	5.6 (4.2, 7.0)	13.0 (10.5, 15.5)	6.6 (4.9-8.6)	10.2 (8.6, 11.7)
	4-5	2.7 (1.9, 3.5)	9.9 (7.6, 12.2)	3.1 (2.3-4.6)	5.1 (4.4, 5.7)
	5-6	0.9 (0.7, 1.1)	9.9 (9.3, 10.4)	1.6 (0.9-2.9)	3.3 (2.7, 3.8)
	0-6	52.1	73.2	47.9	44.7
Total	0-6	52.1	73.2	47.9	44.7
^{14}C content of bile	0-6	2.8 (2.1, 3.4)	10.4 (9.4, 11.4)	3.7 (3.2-4.2)	2.8 (2.5, 3.1)
^{14}C content of respired air	0-6	11.7 (11.6, 11.8)	10.2 (7.3, 13.0)	n.d.	n.d.
Recovery (% of injected dose)		66.6	93.8	51.6	47.5

in these organs was greater than that in the blood. The amount of label in both organs decreased with time and radioactivity could not be detected in radioautograms obtained from animals killed 24h after administration of either surfactant. These findings are in accord with previous quantitative results in demonstrating the facility with which both surfactants are absorbed from the gastrointestinal tract and the peritoneal cavity and implicate the liver as the major site of metabolism.

Qualitative analysis of urine samples and faecal extracts

Faeces were collected for 48h from animals receiving either sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate. The compounds were administered either orally or

intraperitoneally. The radioactive contents of faeces samples were extracted quantitatively with methanol and portions (100 μl) of the extracts subjected to t.l.c. in solvents A and B together with authentic markers. All chromatograms showed the presence of a radioactive component that co-chromatographed with the injected parent surfactant. In addition, faeces obtained from animals injected orally with $[1-^{14}\text{C}]$ undecyltriethoxy sulphate also contained radioactive material that co-chromatographed with one of the radiolabelled urinary components.

Urines were collected at hourly intervals for 6h from animals injected intravenously with either sodium $[1-^{14}\text{C}]$ undecyltriethoxy sulphate or sodium $[1-^{14}\text{C}]$ dodecyltriethoxy sulphate and portions (50-100 μl) were chromatographed together with authentic markers in solvent C. Radiochromatograms

showed that the urines did not contain unchanged parent surfactant, but a mixture of poorly separated metabolites whose chromatographic mobilities suggested increased polarity with respect to the parent compounds.

Purification of urinary metabolites

Before using the purification procedures described, it was established that the chromatographic pattern of urinary metabolites was unaltered when the dose of either surfactant was increased to 20mg/animal.

Rats (four) received either sodium [1-¹⁴C]undecyltriethoxy sulphate or sodium [1-¹⁴C]dodecyltriethoxy sulphate orally at a dose of 20mg/animal. The urines obtained from animals which had received either surfactant were treated identically. Urines were collected for 6h, pooled and diluted to 400ml with water.

Stage I. The diluted urine was passed through a column (12cm × 1cm) of Dowex 1 anion-exchange resin (X4; 50–100 mesh; Cl⁻ form) at a flow rate of approx. 100ml/h. The column was washed with water (20ml) and over 95% of the applied radioactivity was retained by the column. Elution of radioactivity was with a gradient of water/1M-KCl formed from two identical and inter-linked beakers containing water (100ml) and 1M-KCl (100ml). Fractions (5ml) were collected and assayed for ¹⁴C content. A single radioactive peak was obtained at a KCl concn. of approx. 0.4M and those fractions within the peak were pooled and freeze-dried.

Stage II. Freeze-dried fractions were dissolved in the minimum volume of water and desalted on a column of Sephadex G-10 (50cm × 3cm). Fractions contained in the single radioactive peak were combined and freeze-dried.

Stage III. Freeze-dried material from Stage II was dissolved in the minimum amount of water, applied, as streaks, to t.l.c. plates and chromatographed in solvent C. Radioactive areas were located by radioautography and radioautograms showed the presence of three urinary components resulting from the metabolism of [1-¹⁴C]undecyltriethoxy sulphate and four components arising from the metabolism of [1-¹⁴C]dodecyltriethoxy sulphate. The relative amount of radioactivity associated with each component was determined by eluting each appropriate area of silica gel with water (5ml) and determining the radioactive contents of the eluates. The results obtained with the metabolites of [1-¹⁴C]undecyltriethoxy sulphate were as follows: Metabolite A (R_F 0.41), 21.3% of the chromatographed radioactivity; metabolite B (R_F 0.50), 74.9%; metabolite C (R_F 0.59), 3.8%. The corresponding results with the metabolites of [1-¹⁴C]dodecyltriethoxy sulphate were: metabolite I (R_F 0.45), 87.5%, metabolite II

(R_F 0.50) 9.8%; metabolite III (R_F 0.60), 1.7%; metabolite IV (R_F 0.70), 1.0%.

Identification of metabolites

Nuclear-magnetic-resonance spectroscopy. Metabolite I (from [1-¹⁴C]dodecyltriethoxy sulphate), purified to radiochemical homogeneity by repeated t.l.c. and intermediate elution of the silica gel as described above, was analysed in ²H₂O on a n.m.r. spectrometer fitted with a Fourier transform capability. The spectrometer was standardized with 3-trimethylsilyl-1-propanesulphonic acid (sodium salt). The main peak at δ 3.706 was due to the two ethoxylate groups adjacent to the carboxylic acid residue of the alkyl chain. This appears as a singlet because the CH₂ groups are magnetically equivalent and therefore no spin coupling occurs. Smaller peaks at δ 3.844 and δ 4.144 was due to the ethoxylate group adjacent to the sulphate moiety. These methylenes are no longer equivalent owing to the proximity of the sulphate group and, as expected, a 1:2:1 triplet for each methylene was found. The fourth peak at δ 3.946 was due to the carboxylic acid moiety which appeared as a singlet because no adjacent proton bearing groups were present. The spectrum was consistent with the identification of metabolite I as ⁻OC₂CH₂(OC₂H₅)₃OSO₃⁻ [2-(triethoxy sulphate)-acetic acid]. N.m.r. and mass spectra have been deposited as Supplementary Publication SUP 50086 at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K.

Radio-gas-liquid-chromatography. To facilitate analysis of the metabolites by g.l.c., the sulphate moiety was removed by hydrolysis with 0.1M-HCl at 100°C for 6h under N₂. Experiments in which either sodium [1-¹⁴C]undecyltriethoxy sulphate or sodium [1-¹⁴C]dodecyltriethoxy sulphate was hydrolysed with 0.1M-HCl and the hydrolysates subjected to t.l.c., demonstrated complete hydrolysis by 6h. A single radiolabelled product was produced identical in mobility with the parent alkyltriethoxy alcohol demonstrating that the conditions of hydrolysis did not result in cleavage of the ether linkages, an event which was apparent only after 24h hydrolysis. Further, a time-hydrolysis curve of purified metabolite I (originating from [1-¹⁴C]dodecyltriethoxy sulphate) demonstrated complete hydrolysis after 6h and the formation of a single product similar in mobility to metabolite I.

The peak containing radioactivity obtained after desalting of metabolites in urine on Sephadex G-10 was hydrolysed as described above, freeze-dried and converted into the trimethylsilyl derivative by reacting with *NO*-bis(trimethylsilyl)trifluoroacetamide (300 μ l; Pierce Chemical Co., Rockford, IL, U.S.A.) mixed with dimethylformamide (100 μ l).

Portions (1–3 μ l) of solutions containing metabolite derivatives of [1- 14 C]dodecyltriethoxy sulphate were analysed by radio-g.l.c. Two peaks containing radioactivity, corresponding to the two major mass peaks, were recorded (retention times, 9 and 13 min). The ratio of the peak areas was about the same as the ratio of the radioactive contents of metabolites I and II.

Radio-g.l.c. of portions (1–3 μ l) of solutions containing metabolite derivatives of [1- 14 C]undecyltriethoxy sulphate revealed a single radioactive peak which corresponded to the major mass peak (retention time, 11.5 min).

Gas-liquid chromatography and mass spectrometry

A solution containing the derivatives and hydrolysed metabolites of [1- 14 C]dodecyltriethoxy sulphate was analysed by g.l.c.–field-ionization mass spectrometry. The line spectrum for the mass peak corresponding to the major radioactive g.l.c. component strongly suggested the presence of the trimethylsilyl derivative of $^{-}\text{O}_2\text{CCH}_2(\text{OC}_2\text{H}_4)_3\text{OH}$. Evidence of one major ion at m/e 353 corresponds to $(M+1)^+$ and another at m/e 337 corresponds to $(M-\text{CH}_3)^+$.

Discussion

Both the surfactants [1- 14 C]undecyltriethoxy sulphate and [1- 14 C]dodecyltriethoxy sulphate are extensively metabolized in the rat with the elimination of the majority of the excreted radioactivity in the urine. However, expired air was a significant route of elimination of ^{14}C when [1- 14 C]undecyltriethoxy sulphate was administered. Virtually all the injected radioactivity was recovered within 48 h of administration of either compound. In general the proportions of radioactivity appearing in urine and respired air after the administration of either surfactant were independent of the route of administration whether oral, intraperitoneal or intravenous. Both surfactants were readily absorbed from the gastrointestinal tract after oral administration although small amounts of unchanged material were detected in the faeces. In this connection, it is interesting to note that after intraperitoneal administration of sodium [1- 14 C]dodecyltriethoxy sulphate to male and female rats and sodium [1- 14 C]undecyltriethoxy sulphate to male rats, considerable proportions of the radioactive dose were eliminated in the faeces, although the significance of the transport of the surfactants from the intraperitoneal cavity into the gastrointestinal tract is not clear at the present time.

Whole-body radioautography strongly implicated the liver as the major site of metabolism. The major urinary metabolite of [1- 14 C]dodecyltriethoxy sulphate was identified as $^{-}\text{O}_2\text{CCH}_2(\text{OC}_2\text{H}_4)_3\text{OSO}_3^{-}$,

a structure which is indicative of degradation by ω,β -oxidation. Such oxidative degradation has been established in the mammalian metabolism of other alkyl anionic surfactants containing both odd- and even-numbered carbon chains (Denner *et al.*, 1969; Burke *et al.*, 1975, 1976; Merits, 1975; Taylor *et al.*, 1976). However, whereas β -oxidation of alkyl sulphates and alkyl sulphonates with even-numbered carbon chains yields the four-carbon sulphate or sulphonate derivative of butyric acid, the alkyl chain of [1- 14 C]dodecyltriethoxy sulphate is degraded so that only a two-carbon fragment of the alkyl chain remains. It appears that the triethoxy moiety acts as a spacer between the alkyl chain and sulphate group negating any steric effect exerted by this group and is itself not susceptible to β -oxidation. The other minor metabolites detected in urine after the administration of sodium [1- 14 C]dodecyltriethoxy sulphate are less polar than 2-(triethoxy sulphate)acetic acid and it is suggested that they are higher homologues of the major metabolite, produced at various stages of β -oxidation of the surfactant.

The major metabolite of [1- 14 C]undecyltriethoxy sulphate in urine was tentatively identified as 3-(triethoxy sulphate)propionic acid on the basis of its behaviour on g.l.c. relative to 2-(triethoxy sulphate)-acetic acid. Thus the odd-numbered carbon-chain triethoxy sulphate surfactant is also degraded by ω,β -oxidation.

Although both surfactants are degraded by the ω,β -oxidation system there is a marked difference in the overall distribution of the label after metabolism of the two compounds. With [1- 14 C]dodecyltriethoxy sulphate small amounts of the label appear in respired air, whereas with [1- 14 C]undecyltriethoxy sulphate substantial proportions of ^{14}C were eliminated via this route. It is possible that this difference may be due to the nature of the major metabolite formed from the latter surfactant. Removal of a further two-carbon fragment from 3-(triethoxy sulphate)propionic acid would yield (triethoxy sulphate)formic acid the inherent instability of which may well be the cause of $^{14}\text{CO}_2$ elimination in considerable amounts. An alternative explanation for the increased liberation of $^{14}\text{CO}_2$ from the odd-numbered carbon chain surfactant is cleavage of the molecule or its metabolites at the ether link proximal to the alkyl chain. It seems unlikely that the undecyl homologue would have greater affinity for such an enzyme system than the dodecyl homologue. The small amounts of ^{14}C recovered in respired air in experiments with the dodecyl derivative may well arise by such an enzyme system capable of hydrolysing ether links as described by Pfeleger *et al.* (1967). However, the amounts of $^{14}\text{CO}_2$ liberated from the undecyl derivative could arise either because of the greater affinity of this compound for an enzyme that hydrolyses the ether

link, or by decarboxylation (enzymic or non-enzymic) of its metabolite(s). It is not possible, at the present time, to distinguish between these alternatives because the suitability of the surfactants and their metabolites for an enzyme system that cleaves ether linkages is not known. Tietz *et al.* (1964) have reported that such an enzyme system shows maximal activity for alkyl glycerol ethers containing 14–18 carbon atoms in the alkyl chain. In addition, McDermott *et al.* (1975) showed that after the administration of [$1-^{14}\text{C}$]hexadecyltriethoxy sulphate to rats or man, some (11 %) of the dose was exhaled as $^{14}\text{CO}_2$. However, in this study the predominant degradative route was ω,β -oxidation since the major metabolite was identified as 2-(triethoxy sulphate)acetic acid. In the present study it has been shown that the biodegradation of both sodium undecyltriethoxy sulphate and sodium dodecyltriethoxy sulphate in the rat also proceeds mainly by ω,β -oxidation although cleavage of the ether link proximal to the alkyl chain represents a minor alternative pathway.

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