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THE FATE OF LYSERGIC ACID DI[^{14}C]ETHYLAMIDE ([^{14}C]LSD) IN THE RAT, GUINEA PIG AND RHESUS MONKEY AND OF [^{14}C]iso-LSD IN RAT

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Abstract—The qualitative and quantitative aspects of the metabolism and elimination of [^{14}C]LSD in the rat, guinea pig and rhesus monkey have been investigated. Rats given an i.p. dose (1 mg/kg) excreted 73% of the ^{14}C in the faeces, 16% in the urine and 3.4% in the expired air as $^{14}CO_2$ in 96 hr. Guinea pigs similarly dosed, excreted 40% in the faeces, 28% (urine) and 18% (expired $^{14}CO_2$) in 96 hr. Rhesus monkeys (0.15 mg/kg i.m.) eliminated 39% of the ^{14}C in the urine and 23% in the faeces in 96 hr.

Extensive biliary excretion of [^{14}C]LSD occurred in both the rat and guinea pig. Bile duct-cannulated rats excreted 68% of an i.v. dose (1.33 mg/kg) in the bile in 5 hr and the guinea pig 52% in 6 hr.

[^{14}C]LSD is almost completely metabolised by all three species and little unchanged drug is excreted. The metabolites identified were 13- and 14-hydroxy-LSD and their glucuronic acid conjugates, 2-oxo-LSD, de-ethyl LSD and a naphthostyril derivative. There occur, however, important species differences in the nature and amounts of the various metabolites. In the rat and guinea pig the major metabolites were the glucuronic acid conjugates of 13- and 14-hydroxy-LSD which were found in both urine and bile. The guinea pig excreted significant amounts of 2-oxo-LSD in urine and bile. De-ethyl LSD was a minor urinary metabolite in both species.

The metabolism of LSD appeared to be more complicated in the rhesus monkey. The urine contained at least nine metabolites of which four were identified as follows: 13- and 14-hydroxy-LSD (as glucuronic acid conjugates) de-ethyl LSD and a naphthostyril derivative. Unlike the rat and guinea pig the glucuronic acid conjugates of 13- and 14-hydroxy-LSD were only present in small amounts. Of the remaining five unidentified metabolites, three were major.

The biliary metabolites of [^{14}C]iso-LSD in the rat have been studied and been shown to be similar to those produced from [^{14}C]LSD, namely 13- and 14-hydroxy-iso-LSD and their glucuronic acid conjugates and 2-oxo-iso-LSD.

In the preceding paper [1] some of the metabolites of (+)-lysergic acid diethylamide (LSD) formed in the isolated perfused rat liver were identified. In this paper the *in vivo* metabolism of LSD is described in the rat, guinea pig and rhesus monkey. Marked differences in the metabolism and excretion were observed in these species. The fate of (+)-iso-LSD was also studied in the rat since it had been suggested by Slaytor and Wright [2] that one of the major metabolites of LSD in this animal was a glucuronide of a hydroxy iso-LSD.

MATERIALS AND METHODS

Compounds

The compounds examined in this paper and their chromatographic characteristics are described in the preceding paper [1] with the exception of the iso-LSD reported below.

(+)-Isolysergic acid di[^{14}C]ethylamide ([^{14}C]iso-LSD). This substance was obtained as a by-product during the synthesis of [^{14}C]LSD [3] and was purified by t.l.c. on silica gel (see preceding paper) in acetone–toluene–aq.NH_3 (sp. gr. 0.88) (100:20:1, by vol.). Both iso-LSD and LSD have an R_f value of 0.36 in this solvent whereas imidazole had an R_f of 0.15. Elution of the mixed amide band and further chromatography in solvent D (see Table 1 of preceding paper) separated the iso-LSD from the LSD (R_f 0.19 and 0.53 respectively) and after elution from the silica gel the iso-LSD was isolated as the maleate salt by dissolving the free base in ether and adding a solution of maleic acid in ether dropwise until precipitation was complete. The radiochemical purity was greater than 95 per cent as estimated by t.l.c. in solvent D, cutting the plate into 5 mm wide sections and scintillation counting. The compound also ran as a single radioactive spot in $CHCl_3$–MeOH (3:2 by vol., R_f 0.40). The mass spectrum was identical with that of an authentic sample of LSD and the specific radioactivity was 13.0 μCi/mg.

Animals

Female Wistar albino rats (150–190 g), female

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Dunkin–Hartley albino guinea pigs (260–360 g) and a male (5 kg) and a female (6 kg) rhesus monkey (*Macaca mulatta*) were used. The compound to be administered was dissolved in (+)-tartaric acid solution (0.5% w/v) and given intraperitoneally to the rats and guinea pigs and intramuscularly to the monkeys. For the monkeys, the solution before injection was sterilised by filtration through a Millex disposable filter unit (0.22 μm; Millipore S.A., France). The rats and the guinea pigs were kept singly in metabolism cages (Metabowl; Jencons, Hemel Hempstead, Herts., U.K.) whereby urine and faeces could be collected separately.

Monkeys were housed in cages with a suitable tray for the separate collection of urine and faeces. The tray and the urine container were treated with aqueous mercuric chloride solution (1% w/v) to prevent bacterial action on the excreta. All the animals were allowed free access to water and kept on an appropriate diet. Food, however, was withheld during the first 24 hr after dosing. Urine and faeces were collected daily.

Male chinchilla rabbits (1.5–3.0 kg) were used for electroencephalographic (EEG) studies and were maintained on a diet of RAF pellets (Labsure Animal Diets, Poole, Dorset, U.K.). The compounds, dissolved in physiological saline containing 0.05% (w/v) (+)-tartaric acid, were administered (77 nmole/kg) intravenously via a cannula in the marginal ear vein.

For the EEG studies hypodermic needles were placed subdermally in the scalp and attached to the leads of the recorder (Elema-Schonander Mingograf EEG 16 Electroencephalograph; Sierex Ltd., Wembley, Middx., U.K.) to produce two channel bipolar recordings giving right and left antero-posterior tracings. A fifth electrode placed at the back of the head acted as an earth.

Animals with biliary fistulae. Cannulation of the common bile duct was carried out as described by Abou-El-Makarem *et al.* [4]. The cystic duct was tied to ensure that hepatic bile only was collected. The LSD, dissolved in the tartaric acid solution, was administered intravenously (i.v.) (femoral vein) to anaesthetised rats and intraperitoneally (i.p.) to anaesthetised guinea pigs. Bile from guinea pigs was collected hourly for 6 hr and, where necessary, further doses of pentothal were given to maintain anaesthesia.

Concentration of metabolites

Pooled freeze-dried bile was extracted with methanol. The extract was taken to dryness under reduced pressure and the residue redissolved in water and chromatographed on XAD-2 as described in the preceding paper [1].

Monkey urine was concentrated directly on XAD-2, as described by Mulé *et al.* [5].

Chromatography

See preceding paper (Siddik *et al.* [1]) for details.

Radiochemical techniques

Radiochemical techniques were those used in the preceding paper, urine being counted in a similar way to bile. Faeces were counted, after homogenisation in 10 parts of water with an Ultra-Turrax homogeniser (Janke and Kunkel A.G., Staufen i. Breisgau, W. Germany), as a suspension in Bray's scintillant containing 5% (w/v) Cab-O-Sil (thixotropic gelling agent, Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.).

For estimation of $^{14}CO_2$, the expired air from the rats and guinea pigs was drawn through a series of three Drechsel bottles. The first contained $CaCl_2$ granules to remove moisture, and the next two contained 250 and 150 ml, respectively, of CO_2 absorbant consisting of 2-methoxyethanol and ethanolamine [6]. This absorbant (3 ml) was added to scintillant (15 ml) prepared as described previously [6]. The absorbant was changed after 8 hr, and then every 24 hr but the $CaCl_2$ was renewed twice daily.

Enzyme treatment

Conjugates in bile and urine were hydrolysed enzymically as described in the preceding paper [1].

Identification of metabolites

In the rat after [^{14}C]*LSD.* Chromatography of the first 5 hr bile and the first 24 hr urine or faecal homogenate on paper in solvent systems A, B and C revealed several metabolites (Table 3). Bile showed a similar pattern of metabolites as that seen with bile from the perfused liver except that no LSD was detected. Further, R4 aglycone, not found in bile from perfused liver, was present and was identified by comparison of R_f values with the product of enzymatic cleavage of R4. Radiochromatograms of urine from intact animals showed a pattern similar to that of bile but metabolite R2 was absent whereas R7, seen in the homogenate of perfused liver, was present. Some six compounds were detected in the homogenate of faeces from intact rats, on paper chromatograms in systems A and C. Metabolites R4, R5 and aglycone of R4, found in rat bile, were also present in the faeces together with three other compounds, R12, R13 and R14.

Metabolites R4 and R5 from bile were found to be the same as the two glucuronides described in the previous paper, using the same methods of identification i.e. glucuronides of 13- and 14-hydroxy-LSD respectively. Metabolites R1-R3 and R6, found in bile, and R7, found in urine, were also the same as those present in the perfused liver by comparison of chromatographic properties and where possible, colour reactions. R14 found in the faeces, appeared to be the decomposed aglycone of R5 since the decomposition of the latter gave a product which had the same R_f as R14 in systems A and C. The identification of R12 and R13 was not further pursued. Metabolite R8 (nor-LSD) identified in the liver perfusion experiments was not found *in vivo*.

In the rat after [^{14}C]*iso-LSD.* Bile from bile duct-cannulated rats receiving [^{14}C]-iso-LSD was paper chromatographed in the three solvent systems A, B and C (Siddik *et al.* [1]). Six ^{14}C peaks were present on the radiochromatograms (Table 4). After treatment of the bile for 2 hr with β-glucuronidase followed by t.l.c. in system E, the ^{14}C peaks corresponding to R18 and R19 diminished in intensity. Thus it appeared that the major biliary metabolites of iso-LSD were glucuronides. Concentration of the metabolites on an XAD-2 column and t.l.c. of the methanol eluate in system E revealed that the glucuronides R18 and R19 had the same fluorescence under u.v. light and colour reactions with Van Urk reagent as the two glucuronides R4 and R5, of LSD.

R20 behaved like the LSD metabolite, R6. Both compounds existed in two isomeric forms both of which were resolvable on paper in system B and by t.l.c. in system E (Table 4). By analogy with the metabolism of LSD, R18, R19 and R20 were probably 13- and 14-hydroxy-iso-LSD glucuronides and 2-oxo-iso-LSD, respectively. Iso-LSD metabolites, R15, R16 and R17, probably corresponded to the unidentified LSD metabolites R1, R2 and R3. The 13-hydroxy-iso-LSD, corresponding to aglycone of R4 could not be detected.

In guinea pigs after [^{14}C]*LSD.* Samples, containing about 10^4 d.p.m., of bile or urine collected during the first 4 or 24 hr respectively were chromatographed on paper in the solvent systems A, B and C. At least nine radioactive spots (G1–9) were seen on each radiochromatogram (Table 5). T.l.c. of urine and bile in systems D and F (Table 5) revealed two more metabolites in the urine (G10 and G11) and one of these (G11) in the bile. Incubation of bile with β-glucuronidase for 24–48 hr followed by chromatography in the three paper systems showed that the peaks corresponding to G4 and G5 were considerably diminished, indicating that these two metabolites were glucuronides. Sulphate conjugates, however, were not detected after similar incubations with sulphatase. Concentration of the glucuronides on an XAD-2 column of bile and t.l.c. in system E (R_f 0.41 and 0.49 respectively) allowed visualisation of the two metabolites. Further chromatography in several systems (see Table 5) together with the colour reactions with Van Urk reagent showed them to be identical with the rat metabolites, 13- and 14-hydroxy-LSD glucuronides, respectively.

Metabolite G8 (Table 5) had a weak blue fluorescence and gave a slowly developing yellow colour with Van Urk reagent. This compound appeared to be 2-oxo-LSD since its chromatographic characteristics were identical with those of R6 found in the rat. The two minor compounds, G10 and G11, by comparison of R_f values in systems D and F were the same as de-ethyl-LSD and LSD, respectively. The small amounts of G10 and G11 present, precluded the use of colour reactions and fluorescence characteristics for their identification as was also the case with G1, G2 and G3, although again by comparison of their R_f values with those for the rat metabolites, they appeared to correspond to R1, R2 and R3, respectively.

In the monkey after [^{14}C]*LSD.* Chromatography of the first 24 hr urine on paper in systems, A, B and C and on the t.l.c. plates in systems D and F, revealed ten metabolites (M1–10) of which three (M8, M9 and M10) were found on t.l.c. plates only (see Table 5). M3 and M4 appeared to be the glucuronides of 13- and 14-hydroxy-LSD respectively, on comparison of their R_f values with the corresponding rat metabolites. Since the amounts of these compounds were very small, they could not be characterised by colour reactions and this was the case for all the other urinary metabolites in the monkey except M7.

Metabolite M7 was the major metabolite in the urine. It was separated from pooled urine with XAD-2 resin and then t.l.c. in solvent D, and further purification was achieved by t.l.c. in system A (R_f 0.29). This metabolite quenched the fluorescence of the silica gel and gave a slowly developing yellow colour with Van Urk reagent, but it was not 2-oxo-LSD since its R_f values were quite different. Neither was it a phenol, since, like LSD, it gave a very pale brown colour with diazotised 4-nitroaniline. When the metabolite (approx. 10 μg) was treated with nitrous acid followed by *N*-(1-naphthyl) ethylenediamine [7], a pink colour was obtained. However, no colour was obtained with LSD, de-ethyl LSD or 2-oxo-LSD similarly treated. This colour reaction indicated that M7 contained a primary aromatic amino group, but attempts at obtaining its mass spectrum were unsuccessful.

M8 co-chromatographed in systems D and F with de-ethyl-LSD suggesting that it was, like R7 and G10, de-ethyl-LSD. When the urine (5 ml) was made basic with 1M-NaOH (0.5 ml), saturated with NaCl and then extracted with heptane–isoamyl alcohol (98:2 v/v; 25 ml), and the extract chromatographed on t.l.c. plates in solvent A, only M9 and M10 were present (R_f 0.42

Table 1. Excretion of ^{14}C after administration of [^{14}C]LSD

Animal (no., sex)	Time after dosing (hr)	Cumulative ^{14}C excreted (% of dose)			
		Urine	Faeces	Expired air as CO_2	Total
Rat (3F)	8	—	—	2.7(2.0–3.3)	—
	24	12.7 (8.1–16.3)	62.2(41.6–82.8)	3.1(2.3–3.6)	78.0
	48	14.7(10.6–17.1)	72.1(64.1–87.6)	3.2(2.4–3.8)	90.0
	72	15.8(12.9–17.4)	72.8(65.2–88.0)	3.3(2.5–3.9)	91.9
	96	16.1(13.4–17.5)	73.1(65.5–88.3)	3.4(2.6–4.0)	92.6
Guinea pig (3F)	8	—	—	12.6(11.6–13.4)	—
	24	23.5(20.9–26.5)	9.1(2.5–17.4)	16.5(15.2–17.7)	49.1
	48	26.3(22.8–29.5)	34.0(29.7–38.2)	17.5(16.2–18.7)	77.8
	72	27.6(23.6–30.1)	39.5(37.3–41.2)	17.7(16.4–19.0)	84.9
	96	27.8(23.8–30.2)	40.3(38.6–41.6)	17.8(16.4–19.2)	85.9
Rhesus monkey (1F, 1M)	24	35.6, 35.5	11.6, 3.1	—	47.2, 38.6
	48	37.5, 38.0	19.5, 15.3	—	57.0, 53.3
	72	37.7, 38.7	22.8, 20.0	—	60.5, 58.7
	96	37.9, 39.1	24.1, 21.5	—	62.0, 60.6

[^{14}C]LSD was administered, dissolved in aqueous (+)-tartaric acid solution (0.5% w/v). Each rat received 1.0 mg/kg and 2.6 μCi per animal, i.p.; each guinea pig, 1.0 mg/kg and 4.1 μCi, i.p.; and each monkey 0.15 mg/kg and 10 μCi, intramuscularly. Urine, faeces and expired air were collected with the rats and guinea pigs; urine and faeces only with the two monkeys. Average values are given with ranges in parentheses; individual values are given for the two monkeys.

Table 2. The biliary excretion of ^{14}C by animals given either [^{14}C]LSD or [^{14}C]iso-LSD

Animal (no., sex)	Compound	Time (hr)	Cumulative ^{14}C excreted (% of dose)		
			Bile	Urine	Faeces
Rat (3F)	[^{14}C]LSD	1	52.1(49.7–55.8)	—	—
		2	63.0(55.4–68.9)	—	—
		3	66.1(57.7–72.1)	—	—
		4	67.4(59.5–73.2)	—	—
		5	67.9(59.9–73.7)	—	—
		24	70.9(62.9–76.9)*	7.4(4.0–12.6)*	0.7(0.0–1.6)*
Rat (3F)	[^{14}C]iso-LSD	1	39.1(36.7–44.3)	—	—
		2	54.1(51.6–59.3)	—	—
		3	61.7(58.8–65.8)	—	—
		4	65.8(62.9–69.2)	—	—
		5	67.9(64.4–71.1)	—	—
		24	74.4(71.1–76.8)†	8.3(4.0–11.5)†	0.8(0.2–1.9)†
Guinea pig (3F)	[^{14}C]LSD	1	30.3(15.7–38.6)	—	—
		2	41.5(27.7–49.2)	—	—
		3	46.4(33.4–53.9)	—	—
		4	48.7(36.0–56.3)	—	—
		5	50.6(38.1–58.0)	—	—
		6	52.1(39.6–59.1)	—	—

* Total, 79.0%.
† Total, 83.5%.
Rats were dosed intravenously with either [^{14}C]LSD, 1.3 mg/kg, 2.8 μCi or [^{14}C]iso-LSD, 1.2 mg/kg, 2.7 μCi and guinea pigs intraperitoneally with [^{14}C]LSD, 0.5 mg/kg, 2.4 μCi. The compounds were dissolved in aqueous (+)-tartaric acid solution (0.5% w/v). Average values are given with ranges in parentheses. Guinea pigs were kept anaesthetised during the bile collection. Rats were kept in restraining cages and allowed free access to water. Animals were kept warm with heating lamps.

and 0.72). M9 co-chromatographed with LSD in systems A, D and F and, since it was extractable with heptane–isoamyl alcohol which Axelrod *et al.* [8] claimed to be specific for LSD, it is probable that M9 was the unchanged drug. M10 had R_f values in the same systems which were consistent with it being the naphthostyril compound of Troxler and Hofmann [9] and corresponded to R9 described in the preceding paper [1].

M1 and M2 corresponded in R_f values on paper to the rat biliary metabolites R1 and R2 (Table 3).

RESULTS

The recovery of ^{14}C after i.p. administration of [^{14}C]LSD to rats and guinea pigs and intramuscular injections into rhesus monkeys is shown in Table 1. Faecal excretion predominated in the rat (73 per cent of the dose in 96 hr) but this was less in the guinea pig and monkey (40 and 23 per cent respectively). However, the ^{14}C excreted in the urine over the same time period was in the reverse order of magnitude, being 16, 28, and 39 per cent respectively in the rat, guinea pig and monkey. The elimination of $^{14}CO_2$ in the expired air was examined only in rats and guinea pigs. In the rat only 3 per cent of the dose was eliminated in the expired air in 96 hr as $^{14}CO_2$ but in the guinea pig nearly 18 per cent was eliminated. In both species most of the ^{14}C in the expired air was excreted in the first 8 hr.

In bile duct-cannulated rats, injected intravenously with either [^{14}C]LSD or [^{14}C]iso-LSD, 73 per cent of the dose was found in the bile in 24 hr, with 8 per cent in the urine and 1 per cent in the faeces (Table 2). When the [^{14}C]LSD was administered i.p. to bile duct-cannulated guinea pigs, 52 per cent of the ^{14}C was excreted in the bile in 6 hr; this is less than found in the rat which excreted 68 per cent in 5 hr.

The two major metabolites in rat bile collected during the first 5 hr were the glucuronides of 13- and 14-hydroxy-LSD (20 and 31% of the dose, respectively). A small percentage of free 13-hydroxy-LSD was also present (2%). The other metabolite in the bile which was identified was 2-oxo-LSD (5%) and there remained three compounds amounting to approx. 8 per cent of the dose which were not identified (Table 3).

The bile of rats given [^{14}C]-iso-LSD contained mainly the glucuronides of hydroxy metabolites. However, unlike LSD, the amount of the glucuronide of 13-hydroxy-iso-LSD (25% of the dose) exceeded that of 14-hydroxy-iso-LSD (14%). The other significant metabolite in the bile was 2-oxo-iso-LSD (10%). As in the case of LSD, there were three compounds (15% of the dose) which were not identified (Table 4).

Faeces collected from rats during the first 24 hr after dosing with [^{14}C]LSD contained a product (31% of ^{14}C dose) which was probably decomposed 14-hydroxy-LSD; 13-hydroxy-LSD was also found to be present both in the free and conjugated state (8% and 3%). The amounts of the glucuronides in the faeces were much smaller than in the bile, suggesting that deconjugation (probably bacterial) had occurred in the gut to give 13-hydroxy-LSD and R14 (Table 3).

In rat urine, as in the bile, the major LSD metabolites were glucuronides of 13- and 14-hydroxy-LSD (6% and 4% respectively). The minor metabolites present were 13-hydroxy-LSD (1%), 2-oxo-LSD (1–2%) and

Table 3. Quantitative aspects of the metabolism of [^{14}C]LSD in the rat

Metabolite	Intact rats (% dose in 24 hr) Urine	Faeces	Cannulated rats (% dose in 5 hr bile)	Colour with: U.v. light	Van Urk reagent	Probable identity
LSD	—	—	—	dark blue	purple → dark blue	
R1	0.1(0.0–0.2)	—	0.9(0.6–1.3)	none	none	unknown
R2	—	—	4.0(2.9–5.1)	none	none	unknown
R3	0.4(0.2–0.7)	—	3.5(2.3–4.5)	none	none	unknown
R4	5.5(3.7–7.1)	2.5(1.6–3.1)	20.3(15.7–24.2)	light blue	immediate light blue	glucuronide of 13-hydroxy-LSD
R4 Aglycone	0.8(0.6–1.0)	7.9(5.3–10.5)	1.6(1.4–1.8)	light blue	immediate light blue	13-hydroxy-LSD
R5	3.5(2.1–5.0)	6.1(4.3–8.6)	31.4(29.2–33.9)	dark blue	mauve → dark blue	glucuronide of 14-hydroxy-LSD
R6	1.5(1.0–1.8)	—	5.0(4.0–7.0)	pale blue	yellow	2-oxo-LSD
R7	0.5(0.2–0.8)	—	—	dark blue	purple → dark blue	de-ethyl-LSD
R12	—	7.1(4.8–9.6)	—	none	none	unknown
R13	—	3.8(3.1–4.3)	—	none	none	unknown
R14	—	31.0(20.6–42.0)	—	none	none	unknown
Sum	12.3(7.8–15.7	58.4(40.7–76.5)	66.7(58.3–74.5)			

For doses and administration of compounds, see Tables 1 and 2. For details of chromatography, see Table 1 of preceding paper [1]. Metabolites were determined by scintillation counting of paper chromatograms (see text). Average values are given with ranges in parentheses.

R12, R13 and R14 had R_f of 0, 0.76 and 0.95 respectively, in paper system C and R12 and R14, 0 and 0.80, respectively, in system A.

The R_f values of the other metabolites were the same as in the preceding paper as were the identities.

R14 was probably decomposed 14-hydroxy-LSD.

The yellow colour of metabolite R6 appeared in about 15 min.

de-ethyl-LSD (0.5%), the latter being only detected in the urine. Nor-LSD, the naphthostyril compound, LSD and lumi-LSD were not found in the excreta of the intact or bile duct-cannulated rat.

In the guinea pig the metabolites were somewhat different from those in the rat. The two major metabolites in the bile were the 14-hydroxy-LSD glucuronide (15% of the dose in 4 hr) and 2-oxo-LSD (14%), together with about 3 per cent of 13-hydroxy-LSD glucuronide. Unchanged LSD, which was not found in the rat, was present in the bile of two of the three guinea pigs examined (see Table 5). Six other biliary metabolites (G1, G2, G3, G6, G7 and G9, amounting to about 13% of the dose) were not identified.

In the urine of the guinea pig collected for 24 hr after dosing 2-oxo-LSD, 13- and 14-hydroxy-LSD account for 3, 1 and 4 per cent of the dose respectively. LSD (0.6%) and de-ethyl-LSD (0.6%; this was not detected in the bile) were present in the urine in very small amounts. Six other compounds representing the major part (12% of the dose) of the ^{14}C in the urine were not identified.

Table 4. R_f values and quantitation of the metabolites of [^{14}C]iso-LSD in rat bile

Metabolite	R_f in solvent: A	B	C	E	Per cent dose in 5 hr bile	Colour with: U.v. light	Van Urk reagent	Probable identity
Iso-LSD	0.84	0.85	0.92	0.62	—	dark blue	purple → dark blue	
R15	—	—	0	—	3.6(2.3–4.1)	none	none	unknown
R16	0.17	0.06	0.06	—	6.4(2.4–9.2)	none	none	unknown
R17	0.28	0.27	0.17	—	5.5(4.2–7.6)	none	none	unknown
R18	0.37	0.36	0.36	0.20	25.3(20.5–28.4)	light blue	immediate light blue	glucuronide of 13-OH-iso-LSD
R19	0.37	0.44	0.45	0.28	14.2(13.8–14.6)	dark blue	purple → dark blue	glucuronide of 14-OH-iso-LSD
R20	0.82	0.74 0.83	0.83	0.52 0.58	9.8(7.6–11.8)	none	none	2-oxo-iso-LSD
R18 aglycone	—	—	—	0.52	—	light blue	immediate light blue	13-OH-iso-LSD
R19 aglycone	—	—	—	0.52	—	dark blue	purple → grey-blue	14-OH-iso-LSD
Sum					64.8(62.4–67.1)			

See Table 3 for details. Paper chromatography was used for solvent systems A, B and C and t.l.c. for system E.

Table 5. R_f values and quantitation of the metabolites of [^{14}C]LSD in the guinea pig and rhesus monkey, G1–11 and M1–10 respectively

	R_f in solvent: A	B	C	D	F	per cent dose in 4 hr bile	per cent dose in 24 hr urine	Probable identity
Guinea pig metabolites								
G1	0	0	0	0	0	0.5(0.2–0.8)	0.4(0.2–0.6)	unknown
G2	0.12	0.12	0.05	0	0	1.6(1.2–2.0)	0.4(0.2–0.6)	unknown
G3	0.18	0.22	0.20	0	0	2.4(2.3–2.6)	0.5(0.3–0.6)	unknown
G4	0.27	0.33	0.37	0	0	2.7(1.4–4.3)	1.4(1.3–1.5)	13-OH-LSD glucuronide
G5	0.33	0.40	0.47	0	0	14.8(9.0–18.8)	3.5(1.4–5.0)	14-OH-LSD glucuronide
G6	0.55	0.53	0.57	0	0	2.7(1.9–3.5)	3.9(2.4–4.8)	unknown
G7	0.76	0.73	0.75	0.16	0.04	1.3(0.7–1.7)	2.8(2.1–3.2)	unknown
G8	0.81	0.73 / 0.87	0.84	0.30	0	14:1(11.8–16.6)	2.5(2.2–2.9)	2-oxo-LSD
G9	0.76	0.73	0.84	0.30	0.14	4.6(2.6–5.9)	3.7(3.1–4.6)	unknown
G10	—	—	—	0.37	0.29	—	0.6(0.2–0.9)	de-ethyl-LSD
G11	—	—	—	0.53	0.47	0.4(0–0.9)	0.6(0.4–0.7)	LSD
Sum						45.1(32.7–52.1)	20.3(17.3–23.2)	
Monkey metabolites								
M1	—	—	0	0	0	—	1.3, 0.8	unknown
M2	—	0.17	0.08	0	0	—	1.7, 2.4	unknown
M3	0.27	0.33	0.37	0	0	—	1.6, 2.7	13-OH-LSD glucuronide
M4	0.33	0.40	0.47	0	0	—	2.3, 3.4	14-OH-LSD glucuronide
M5	0.63	0.60	0.81	0	0	—	5.2, 6.1	unknown
M6	0.77	0.72	0.75	0.16	0.07	—	5.7, 5.0	unknown
M7	0.77	0.72	0.85	0.31	0.16	—	11.4, 11.2	unknown
M8	—	—	—	0.39	0.33	—	1.1, 0.6	de-ethyl-LSD
M9	—	—	—	0.53	0.48	—	1.6, 1.0	LSD
M10	—	—	—	0.68	0.55	—	1.2, 0.6	naphthostyril compound
Sum							33.1, 33.8	

See Tables 1, 2 and 3 for details of dosing and chromatography. Paper chromatography was used for solvent systems A, B and C and t.l.c. for systems D and F.

The metabolism of LSD in a male and a female rhesus monkey was similar, but different from that in the rat and guinea pig. The urine collected during the first 24 hr after dosing contained unchanged LSD (M9; 1–2% of the dose), de-ethyl-LSD (M8; approx. 1%) and the glucuronide conjugates of 13- and 14-hydroxy-LSD (M3 and M4 about 2–3% of each), but the major metabolite (M7; 11%) was not identified nor were four other products (M1, M2, M5, M6) which together represented 14 per cent of the dose. 2-Oxo-LSD was not detected, but its degradation product, the naphthostyril compound (M10), was present in the urine (approx. 1%) (Table 5). 13-Hydroxy-LSD, nor-LSD and lumi-LSD were not found in monkey urine.

EEG studies

Synthetic and biosynthetic metabolites of LSD were injected intravenously into conscious restrained male chinchilla rabbits. With LSD itself, de-ethyl-LSD, 12-hydroxy-LSD, 12-methoxy-LSD, 13-hydroxy-LSD, 13-methoxy-LSD and 13-hydroxy-LSD glucuronide, a persistent alerting EEG trace was seen as indicated by an increase in frequency and decrease in amplitude of the waveform. No changes were observed after administration of lysergic acid, di-LSD-disulphide [10], nor-LSD, 14-hydroxy-LSD-glucuronide, 14-methoxy-LSD, lumi-LSD or the metabolic 2-oxo-LSD.

DISCUSSION

In Table 6, the extent of the excretion of the ^{14}C of [^{14}C]LSD by various routes in the rat, guinea pig and rhesus monkey has been summarized and a clear species difference in the amount of ^{14}C eliminated by these routes, urine, faeces, expired air and bile, is evident. The biliary excretion of ^{14}C in the rat is some 68 per cent of the dose in 5 hr (68% for iso-LSD) and this is reflected in the faecal excretion of ^{14}C which is 73 per cent. In the guinea pig the biliary excretion is less (51%) as is the faecal excretion (40%). The main metabolites of LSD in the rat and probably the guinea pig are the glucuronides of 13- and 14-hydroxy-LSD (See Table 7), the molecular weight of each of which is 515. According to Millburn *et al.* [11], the biliary excretion of anions begins to become significant in the rat when their molecular weights begin to exceed 325 ± 50, and in the guinea pig when they exceed 400 ± 50. The glucuronides of the hydroxy-LSD's could therefore be expected to be excreted extensively in the bile in the rat and the guinea pig, more so in the rat, because of the lower

Table 6. Comparative values for the routes of excretion of [^{14}C]LSD*

Species	Per cent of dose of ^{14}C excreted by; intact animals in 96 hr: in urine	in faeces	in expired air	Total	bile duct-cannulated animals in 5 hr: in bile
Rat	16	73	3	92	68 (iso-LSD, 68)
Guinea pig	28	40	18	86	51
Rhesus monkey	39	23	n.d.	61	n.d.

* Average values to the nearest whole number, taken from the preceding tables, are given.
n.d. = not determined.

figure for the initiation of extensive biliary excretion, than in the guinea pig. The value of the molecular weight for significant biliary excretion in the rhesus monkey is thought to be higher and about 500 (see Smith [12]), so that one might expect less biliary excretion in the monkey than in the rat and guinea pig, a view which is supported by the lower faecal excretion (23%) of ^{14}C in this animal. Furthermore, in the monkey, the glucuronides of the hydroxy-LSD's may not be major metabolites (see Table 5).

Table 7 shows that in the rat, LSD is metabolized mainly by aromatic hydroxylation, since more than half the dose of LSD is eliminated in the bile as conjugated 13- and 14-hydroxy-LSD. This occurs probably to a lesser extent in the guinea pig although it may be still a major pathway. In the monkey, aromatic hydroxylation occurs but it may be of less importance than other pathways of metabolism which have not been elucidated. The formation of 13- and 14-hydroxy-LSD might suggest an intermediate 13,14-epoxide, in which case a glutathione conjugate of LSD and the corresponding mercapturic acid and a 13,14-dihydrodiol could be metabolites of LSD, but these were not looked for. It is to be noted that the ratio 13-hydroxy-LSD/14-hydroxy-LSD in the rat bile (0.64) is much greater than that in the guinea pig bile (0.2), whilst for iso-LSD the ratio is 1.8. These ratios suggest that the guinea pig is a poor hydroxylator of the 13-position compared with the rat, and that when iso-LSD is compared with LSD in the rat, it would appear that in LSD the 14-position is better hydroxylated than the 13, whereas with iso-LSD the reverse is true. Whether these differences have any significance is a matter for further investigation.

Another difference which shows up in table 6 is that the ^{14}C in the expired air of the guinea pig (18%) is six times that in the rat (3%). This suggests that the de-ethylation of LSD occurs more readily in the guinea pig than in the rat and that de-ethyl-LSD, or even lysergic acid amide, should be more readily detected as a metabolite in the guinea pig than in the rat. However, de-ethyl-LSD in similar amounts (0.5% of the dose) was detected in both rat and guinea pig urine. The expired air was not examined in rhesus monkeys, but the recovery of ^{14}C in the urine and faeces was consistently low (61%) in these animals. This could suggest that de-ethylation was more extensive in the monkeys than in the guinea pigs and in fact rather more de-ethyl-LSD (0.9%) was found in monkey urine (Table 7).

A significant metabolite found in guinea pig bile was 2-oxo-LSD. The amount of this compound (14%) was comparable with 14-hydroxy-LSD glucuronide (15%). The 2-oxo-LSD was present to a lesser extent in the rat

Table 7. Comparative values of some metabolites of LSD

Metabolite	Per cent of dose of [^{14}C]LSD: in bile: rat (5 hr)	in bile: guinea pig (4 hr)	in urine in 24 hr: rat	in urine in 24 hr: guinea pig	in urine in 24 hr: rhesus monkey*
13-Hydroxy-LSD glucuronide	20 (iso-LSD, 25)	3	5.5	1.4	2.1
14-Hydroxy-LSD glucuronide	31 (iso-LSD, 14)	15	3.5	3.5	2.9
2-Oxo-LSD	5 (iso-LSD, 10)	14	1.5	2.5	(0.9 naphthostyril)
De-ethyl-LSD	n.d.	n.d.	0.5	0.6	0.9
Unchanged LSD	n.d.	0.4	n.d.	0.6.	1.3

Average values to the nearest two numbers are given.
n.d. = not detected.
* In the rhesus monkey, the main urinary metabolite (M7, see Table 5) is an unknown, amounting to 11 per cent of the dose of ^{14}C or about one third of the ^{14}C excreted in 24 hr.

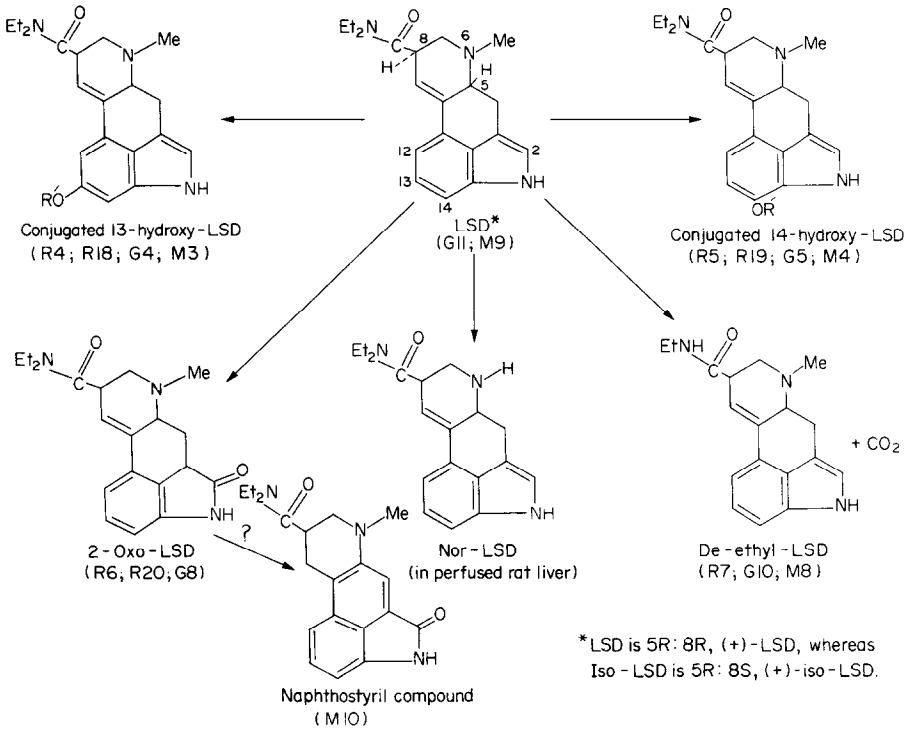

Fig. 1. An outline of the metabolite routes of LSD (and iso-LSD) as suggested from the present study. The metabolites which have been identified are given in parentheses which also identifies the species (i.e. R = rat; G = guinea pig; M = monkey). In the conjugated hydroxy-LSD's, R′ = $C_6H_9O_6$.

bile (5%). This metabolite, however, was found to be present in larger quantities in bile from the isolated perfused liver as described in the preceding paper [1] but this could be due to using higher doses of LSD in this preparation. 2-Oxo-LSD was found in rat urine (1.5%) but in smaller amounts as might be expected, than in guinea pig urine (2.5%). Monkey urine did not contain 2-oxo-LSD, but a naphthostyril compound (1%) derived from it.

Demethylation of LSD at position 6 would give rise to nor-LSD or de-methyl-LSD, but this compound was not detected in the excreta of the rat, guinea pig or monkey, although it has been detected in the isolated perfused rat liver [1]. Failure to detect nor-LSD as a metabolite *in vivo* does not rule out demethylation as a metabolic reaction of LSD, since one or more of the other metabolites which were not identified might well contain the demethylated structure. It is to be noted that nor-LSD and de-ethyl-LSD have been reported by Niwaguchi *et al.* [13] as *in vitro* metabolites of rat, guinea pig and rabbit liver microsomes.

The major metabolite of LSD in the monkey, (M7, Table 5) was not phenolic in nature, but its reactions with Van Urk reagent (yellow) and *N*-(1-naphthyl) ethylene-diamine dihydrochloride (pink) suggest that it might be a product of cleavage of the pyrrole ring of LSD to give an aromatic primary amine resulting from the loss of the 2-carbon as seen with skatole [14], indole [15] and tryptophan [16].

LSD was extensively metabolized in the three species studied and the unchanged drug was not detected in the rat, but small amounts (see Table 7) were found in guinea pig and monkey urine and guinea pig bile. In the case of man, it is also difficult to find any unchanged LSD in the urine of people who have taken the drug (A.C. Moffat, Home Office Central Research Establishment, Aldermaston, Berks, U.K.).

An outline of the routes of metabolism of LSD as delineated in this work is given in Fig. 1. The extent to which LSD is metabolized along these routes is dependent upon species.

Preliminary studies have indicated that some of the metabolites of LSD, as well as the drug itself, produce an activation of the EEG of the conscious rabbit suggesting they may have central activity. These findings will be published elsewhere.

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REFERENCES

1. Z. H. Siddik, R. D. Barnes, L. G. Dring, R. L. Smith and R. T. Williams, *Biochem. Pharmac.* **28**, 3081 (1979).
2. M. B. Slaytor and S. E. Wright, *J. mednl pharm. Chem.* **5**, 483 (1953).
3. R. D. Barnes, *J. labelled Compounds* **10**, 207 (1974).

4. M. M. Abou-El-Makarem, P. Millburn, R. L. Smith and R. T. Williams, *Biochem. J.* **105**, 1289 (1967).
5. S. J. Mulé, M. L. Bastos, D. Jukofsky and E. Saffer, *J. Chromat.* **63**, 289 (1971).
6. H. Jeffay and J. Alvarez, *Analyt. Chem.* **33**, 612 (1961).
7. A. C. Bratton and E. K. Marshall, *J. biol Chem.* **128**, 537 (1939).
8. J. Axelrod, R. O. Brady, B. Witkop and E. V. Everts, *Ann. N.Y. Acad. Sci.* **66**, 435 (1957).
9. F. Troxler and A. Hofmann, *Helv. Chim. Acta* **42**, 793 (1959).
10. K. Freter, J. Axelrod and B. Witkop, *J. Am. Chem. Soc.* **79**, 3191 (1957).
11. P. Millburn, R. L. Smith and R. T. Williams, *Biochem. J.* **105**, 1275 (1967).
12. R. L. Smith, *The Excretory Function of Bile; The Elimination of Drugs and Toxic Substances in Bile.* Chapman & Hall, London (1973).
13. T. Niwaguchi, T. Inoue and Y. Nakahara, *Biochem. Pharmac.* **23**, 1073 (1974).
14. R. A. Heacock and M. E. Mahon, *Can. J. Biochem. Physiol.* **41**, 2381 (1963).
15. L. J. King, D. V. Parke and R. T. Williams, *Biochem. J.* **98**, 266 (1966).
16. A. Meister, *Biochemistry of the Amino Acids*, Vol. 2, 2nd edn. Academic Press, London (1965).