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The Fate of LSD in the Body: Forensic Considerations

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The metabolism of LSD has been studied by analysing plasma and urine samples from Rhesus monkeys treated with radioactively labelled LSD. Plasma ^{14}C radioactivity reached a maximum value within one hour after treatment and declined over the following 23 hours. The ^{14}C radioactivity was detectable in the urine samples for up to 48 hours and fractionation by HPLC *showed seven peaks of radioactivity, five of which cross-reacted in the* RIA *procedure.*

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Introduction

Lysergic acid diethylamide (LSD) is a remarkably potent drug which has its principal effects on the central nervous system. The effective oral dose in man is 0·5–1·0μg/kg. The mechanism of action of the drug is not known, but its pharmacology has been reviewed by Hofmann (1968) and Cohen (1970).

The absorption, distribution and metabolism of LSD in experimental animals has been studied (Axelrod *et al.*, 1957; Stoll *et al.*, 1955; Lanz *et al.*, 1955; Idänpään-Heikkilä and Schoolar, 1969), and our present knowledge of the routes of LSD metabolism in experimental animals, is summarised in Figure 1. Little work has been carried out in man. Upshall and Waillling (1972) found that the plasma concentration of LSD reached a maximum value within one hour of administration; this agreed well with the onset of psychic effects. The half life of LSD in plasma in man, is about 175 minutes. In the only reported study of LSD metabolism in man, Faed and McLeod (1973) were unable to demonstrate the presence in urine of any substances unequivocally derived from LSD.

LSD is not used therapeutically in the United Kingdom but it has been abused to a considerable extent over the past twenty years. No precise figure can be placed on this abuse, but the figures at present are much lower than for cannabis. The detection of LSD in body fluids is difficult because of the very small quantities involved. We have developed a sensitive method for its detection in blood and urine, using a combination of high performance liquid chromatography (*HPLC*) and radioimmunoassay (*RIA*) (Twitchett *et al.*, 1978; Ratcliffe *et al.*, 1977). This method has been applied to cases submitted to operational forensic science laboratories with considerable success. In every instance, there were peaks of cross-reactivity in the *RIA*-chromatograms in addition to that for LSD. If these substances could be shown to be derived from LSD, this would be of considerable forensic importance, as it would provide further evidence of prior possession of the drug. This evidence might also be used to estimate the dose taken and/or when the drug was ingested. Casework samples do not provide suitable material with which to investigate this further, since neither the dose of LSD taken, nor the time between ingestion and obtaining the blood or urine sample are known. It was not possible, for a variety of reasons, to obtain human volunteers to take LSD and provide samples

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of body fluids under controlled conditions; experiments were therefore carried out using Rhesus monkeys, which have been shown to closely resemble man in the way they metabolise many drugs (Smith and Williams, 1974; Williams, 1974).

Materials and Methods

Reagents

^{3}H-LSD: (2(n)–^{3}H) LSD, specific activity 34 mCi/mg; Radiochemical Centre, Amersham, Buckinghamshire.

^{14}C-LSD: Lysergic acid di(^{14}C)-ethylamide, specific activity 72 μCi/mg; Radiochemical Centre, Amersham, Buckinghamshire.

LSD-tartrate was generously supplied by Sandoz Products Ltd., Feltham, Middlesex.

PPO and dimethyl POPOP: Intertechnique Ltd., Uxbridge, Middlesex.

Triton X–100: Koch Light Laboratories Ltd., Colnbrook, Buckinghamshire.

NCS Solubiliser: Amersham/Searle Ltd., High Wycombe, Buckinghamshire.

Ketodase: William R. Warner Ltd., Eastleigh, Hampshire.

β-Glucuronidase (type H2): Sigma (London) Chemical Company Ltd., Kingston-upon-Thames, Surrey.

All other reagents were of analytical grade.

Equipment

Homogeniser: MSE Ltd., Crawley, Sussex.

The liquid scintillation counter used was an Intertechnique SL20 or a Nuclear Enterprises 8312.

The mass spectrometer was a VG Micromass 16F.

Treatment of animals and collection of samples

Adult male Rhesus monkeys were used, weighing 2·8kg–3·5kg (average weight 3·1kg). The monkeys, which were used individually, were sedated with ketamine (6mg/kg) before being placed in a specially constructed cage, designed to allow separate collection of urine and faeces. LSD was administered orally as a solution of the tartrate; the amounts of drug administered to each animal are shown in Table 1. In the experiments where ^{3}H-LSD was administered, unlabelled drug was added so that the total amount administered was approximately the same as in the ^{14}C-LSD experiments; this was necessary because of the different specific activities of the two labelled compounds.

The experimental protocol involved collecting samples of urine and faeces before the administration of LSD, and the collection of further samples as follows: blood: 1ml at 1, 2, 3, 4, 6 and 24 hours after administration; urine: at 1, 2, 3, 4, 6, 24 and 48 hours after administration; faeces: at 24 and 48 hours after administration. In practice it was difficult to obtain 1ml of blood at each time and often much less was collected; also, the supply of urine was erratic.

Determination of radioactivity in samples

Urine. Portions of urine sample (0·2ml) were placed in counting tubes, and

TABLE 1

LSD DOSAGE OF MONKEYS

Animal	*Wt (kg)*	*Dose of LSD* $^{3}H(\mu g)$	$^{14}C(\mu g)$	*μg*	*mg/kg*
171	3·00	—	630	—	0·21
303	2·85	6·6	—	500	0·18
172	3·50	6·6	—	500	0·15
164	2·80	—	—	2856	1·02
170	3·00	—	—	2910	0·97
172	3·40	—	—	3570	1·05

water (0·3ml) and scintillation fluid (4ml) (4g/l PPO, 0·2g/l dimethyl POPOP in toluene containing 33% v/v Triton X-100) were added. The contents of the tubes were mixed thoroughly and allowed to stand in the scintillation counter (at 10°C) for 30 minutes, for dark adaptation to occur. The radioactivity in the tubes was measured by liquid scintillation counting; the amount of quenching was determined by an external standard ratio counting method.

Plasma. Portions of plasma sample (0·05ml) were placed in counting tubes with water (0·45ml) and scintillation fluid (4ml). The radioactivity and degree of quenching were measured as described above.

Faeces. The whole faecal sample was homogenised in 4 volumes of distilled water. Homogenate (0·2ml) and NCS solubiliser (1·2ml) were placed in a counting tube and incubated overnight in an oven at 50°C. Scintillation fluid (4ml) was added, and the radioactivity and degree of quenching determined as described above.

Blood Cells. The blood cells from each sample were suspended in isotonic saline (0·5ml); portions (0·2ml) of the suspension were incubated overnight at 50°C with NCS solubiliser (2·4ml) in a counting tube. Scintillation fluid (4ml) was added, and the amount of radioactivity and degree of quenching determined as above.

Radioimmunoassay of samples

The amount of cross-reacting material in the urine samples from the ^{14}C-LSD-treated and unlabelled LSD-treated monkeys was determined using the radioimmunoassay method (antiserum II) already described (Ratcliffe *et al.*, 1977). In some experiments, it was necessary to measure ^{14}C and ^{3}H simultaneously, using a dual counting method.

Fractionation of urine samples by HPLC

These experiments were carried out on the urine samples from the monkeys which received ^{14}C-labelled LSD. Portions of sample (0·1 or 0·2ml) were chromatographed using the apparatus described by Twitchett *et al.*, (1978). The chromatographic conditions were:

Column:	10cm × 4·6mm i.d. Spherisorb-5-ODS
Eluent:	methanol/aqueous $(NH_4)_2CO_3$(0·1%w/v). 1 : 1 v/v
Flow rate:	1ml/min.

Fractions obtained from these urines were assayed for cross-reactivity by *RIA*, and the amount of ^{14}C-radioactivity was determined by liquid scintillation counting, as described above.

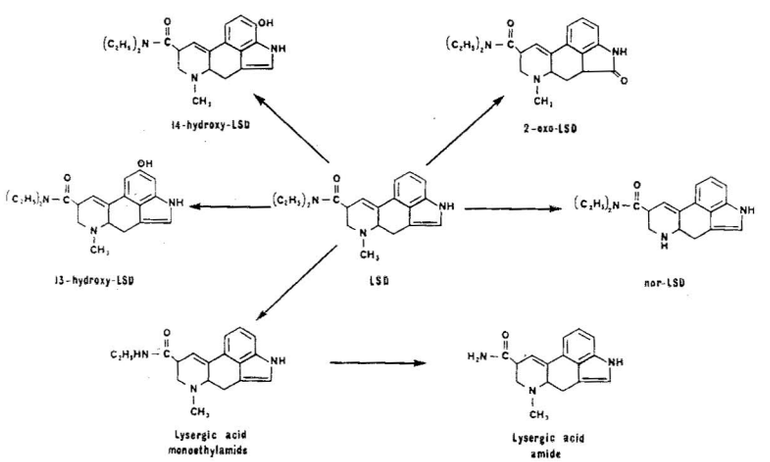

Figure 1. Routes of metabolism of lysergic acid diethylamide.

Enzymic hydrolysis of urine samples

Portions of urine sample (0·1ml) were mixed with acetate buffer (0·2ml, (0·2M, pH5), and either Ketodase (0·2ml, a preparation of β-glucuronidase and sulphatase) or sulphatase (1·0ml). The mixture was incubated for 48 hours at 37°C. At the end of this time, methanol (2ml) was added to precipitate the protein and the suspension was centrifuged to remove the precipitated protein. The supernatant was reduced to a small volume in a stream of nitrogen and fractionated by *HPLC* as described above. The amount of radioactivity in each fraction was determined by liquid scintillation counting.

Identification of cross reacting substances

Experiments were carried out to identify, by mass spectrometry, the components which showed cross reactivity in the *RIA*. Portions (1·0ml) of urine samples from monkeys which had received a high dose of unlabelled LSD were chromatographed as described above, and fractions of eluent were collected. The fractions having cross-reactivity were freeze-dried, the residue taken up in methanol (approximately 50μl), and the whole sample transferred to the probe tube of the mass spectrometer. The conditions used for the analysis were as follows:

Mode:	Electron impact
Emission current:	100μA
Accelerating Voltage:	4kV
Electron energy:	70eV
Source temperature:	150°C
Sensitivity:	$10^{-7}/10^{-8}$; spectra were run in the 'scan down' mode.

Results

Determination of radioactivity in samples

Urine. Figure 2 shows the amount of ^{14}C in each urine sample with time and the cumulative excretion. After 48 hours, 24% of the administered dose had been excreted. The corresponding data for one of the monkeys receiving ^{3}H-LSD are shown in Figure 3; insufficient samples were obtained from the second animal to repeat the experiment.

Plasma. The amount of ^{14}C in the plasma from the monkey which received ^{14}C-LSD is shown in Figure 4A. The plasma concentration reached a maximum value at or before 1 hour, and decreased over the next 23 hours. The data from the two monkeys given ^{3}H-LSD are shown in Figure 4B, in both animals the

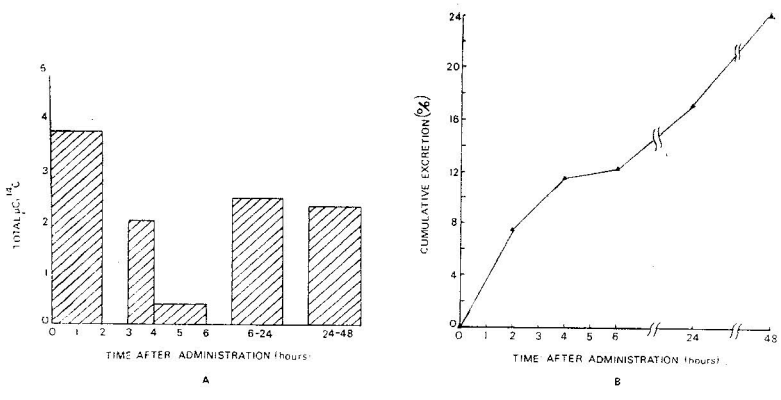

Figure 2. Urinary excretion of ^{14}C after administration of ^{14}C-LSD (45μCi, monkey 171). A: total μCi ^{14}C in each urine sample; B: cumulative excretion of ^{14}C, expressed as % dose administered.

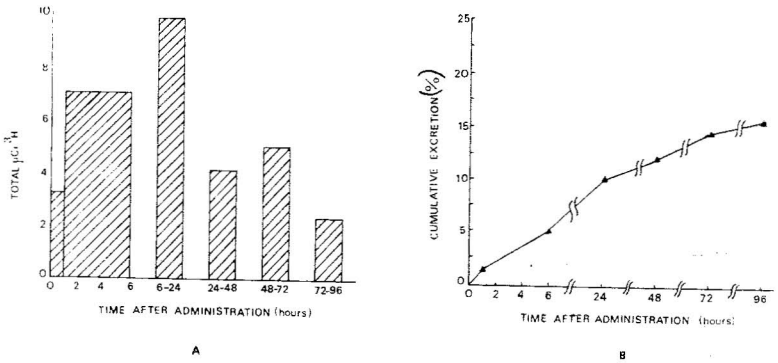

Figure 3. Urinary excretion of ^{3}H after administration of ^{3}H-LSD (225μCi, monkey 303). A: total μCi ^{3}H in each urine sample; B: cumulative excretion of ^{3}H, expressed as % dose administered.

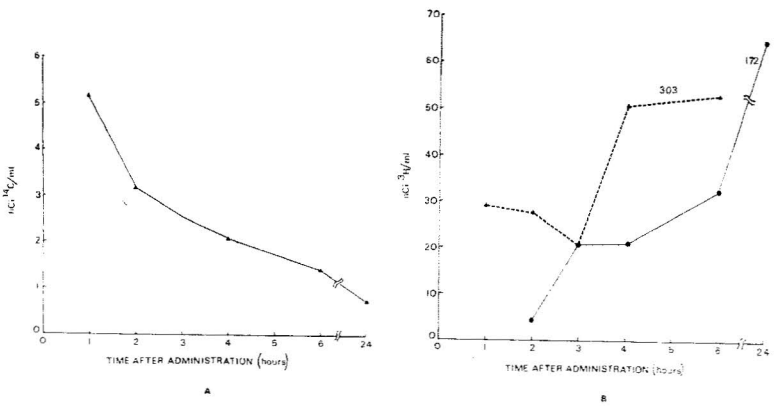

Figure 4. A: Plasma levels of ^{14}C after administration of ^{14}C-LSD (45μCi, monkey 171); B: plasma levels of ^{3}H after administration of ^{3}H-LSD (225μCi; monkeys 172 and 303).

plasma concentration of ^{3}H rose slowly over the duration of the experiment.

Faeces. No ^{14}C or ^{3}H was detected in the faeces of the animals treated with ^{14}C-LSD or ^{3}H-LSD.

Blood Cells. No ^{14}C or ^{3}H was detected in the blood cells of the animals treated with ^{14}C-LSD or ^{3}H-LSD.

Radioimmunoassay of urine samples

Figure 5 shows the cross-reactivity of the urine samples from the monkey that received ^{14}C-LSD; for comparison, the amount of radioactivity in each sample is shown again.

Fractionation of urine samples by HPLC

It was found that 50% methanol–50% $(NH_4)_2CO_3$(0·1% aq) gave a good separation of the various radioactive components in the 0–2 hour urine sample from the monkey which received ^{14}C-LSD. Using this eluent, seven major peaks of radioactivity were obtained (1–7, Figure 6). Figure 7 shows the amount of cross-reactivity in fractions of the same urine; here, five peaks (A–E) of cross reactivity were obtained, corresponding to peaks 1, 3, 4, 6 and 7 in Figure 6.

Effect of enzyme hydrolysis

No difference before and after treatment with sulphatase or β-glucuronidase

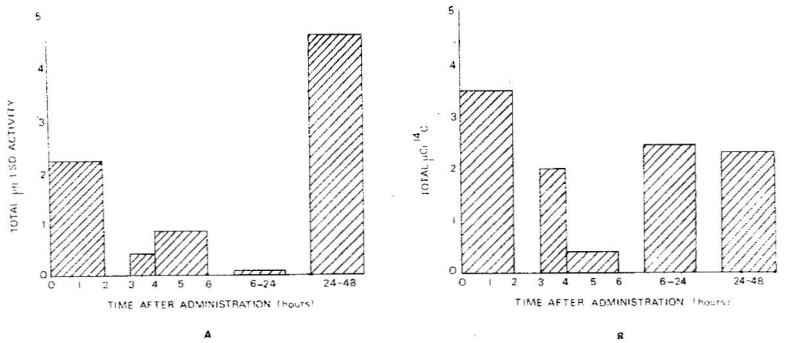

Figure 5. Comparison of urinary excretion of LSD activity (A) and ^{14}C (B) after administration of ^{14}C-LSD (45μCi, monkey 171).

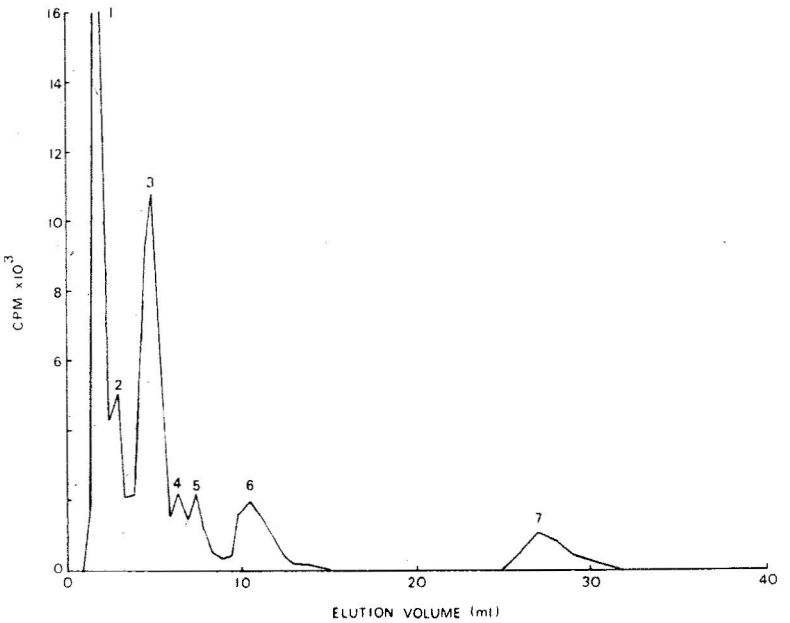

Figure 6. Radiochromatogram of 0–2 hour urine sample from monkey 171 (45μCi ^{14}C-LSD).

was observed in the radiochromatograms of urine from the monkey which had received ^{14}C-LSD.

Identification of cross-reacting substances

It was not possible, in these experiments, to identify by mass spectrometry any compounds related to LSD in any of the fractions obtained after HPLC.

Discussion

Prior to the administration of LSD, the monkeys were given ketamine to facilitate handling. This caused the animals to become sedated and, possibly because of this, no changes in behaviour attributable to LSD were observed. It should be noted, however, that behavioural effects of LSD are very difficult to detect in experimental animals without the use of fairly sophisticated 'psychological' tests (Upshall, 1976). Other workers have reported obvious effects of

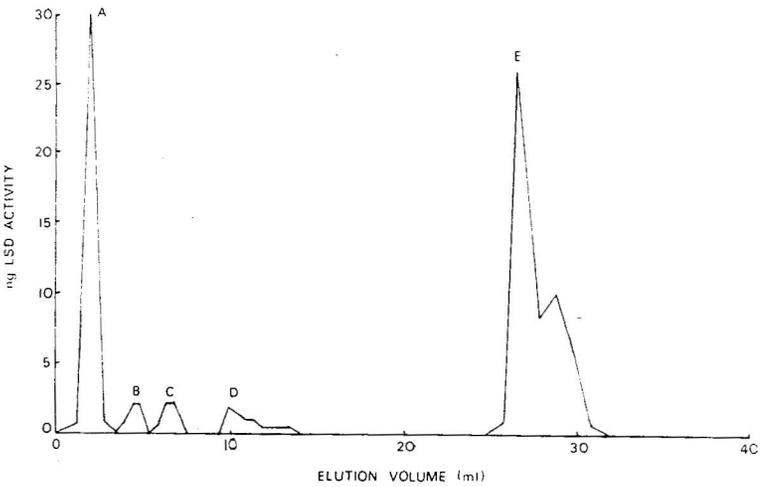

Figure 7. Radioimmunochromatogram of 0–2 hour urine sample from monkey 171 (45μCi ^{14}C-LSD).

LSD on animal behaviour. Evarts (1956) found that monkeys became ataxic after a high dose of LSD, and Laguitina *et al.* (1964) found that LSD caused a reduction of motor activity in baboons, and also a behaviour pattern that they interpreted as evidence of hallucinations.

The results of the present experiments indicate that LSD is absorbed rapidly from the gastrointestinal tract, since the blood level of ^{14}C labelled compounds reached a maximum value within one hour of administration and decreased steadily thereafter. This result is consistent with the findings of Aghajanian and Bing (1964) and of Upshall and Wailling (1972) who found that the plasma level of LSD rose to a maximum level approximately 60 minutes after oral administration. These findings are also in agreement with the fact that the psychic effects of LSD in man are apparent after 40–60 minutes. It was not possible in these experiments, because of the small volume of plasma available, to determine how much of the ^{14}C was present as LSD, and how much was present as metabolites.

The present results further indicate that the main route of excretion of LSD in the Rhesus monkey is via the urine. Over the 48 hours following the administration of the drug, 24% of the administered dose was recovered in the urine, while none was found in the faeces. These results are at variance with the findings of Siddik (1975) who found that in Rhesus monkeys approximately 60% of the administered LSD was excreted within 48 hours, 38% in the urine, and 22% in the faeces. The main differences between the present experiments and those of Siddik are in the dose of LSD given and the route of administration (1mg/kg orally, and 0·1mg/kg intramuscularly respectively.) These differences do not, however, seem sufficient to explain the discrepancy in faecal excretion.

The experiments in which the plasma levels of radioactively labelled compounds were measured at various times after LSD administration showed a marked difference according to the type of label used. In the experiments using LSD containing ^{14}C-ethyl groups on the amide side chain the plasma level of radioactivity reached a maximum in 1 hour, and declined thereafter. In contrast, the experiments using LSD labelled in the '2' position with ^{3}H showed a very different situation. In the first animal used, the blood level of ^{3}H fell slightly over the first two hours, then rose steadily for the duration of the experiment (*i.e.*, up to six hours). These results were so different from those obtained from the ^{14}C monkey that the experiment was repeated. The results of this second experiment showed a steady rise in plasma ^{3}H level over the 24

hours following administration of ^{3}H-LSD, and there was close agreement with the results of the first ^{3}H experiment. A possible explanation of this discrepancy may lie in the lability of the ^{3}H in the '2' position in the LSD molecule. If the label had been lost by an exchange process, it is conceivable that it may have entered the body's general metabolism, primarily in the liver. This could lead to a slow release of ^{3}H labelled substances into the bloodstream, and give rise to the type of picture obtained in these experiments.

Experiments were carried out to investigate the distribution of radioactivity and *RIA* cross-reactivity in the various urinary components. The results obtained indicated that the radioactivity was contained in seven peaks in the chromatogram (1–7, Figure 6); five of these peaks (1, 3, 4, 6 and 7, A–E in Figure 7) also showed cross-reactivity in the *RIA*. Assuming peak E to be LSD, and assuming the specific activity of the components in peaks A, B, C and D to be the same as that of LSD, the degree of cross reactivity has been calculated as: peak A: 10%; peak B: 3%; peak C: 4%; peak D: 6%. This distribution of radioactivity between components in the urine not corresponding to LSD may explain the lack of correlation between radioactivity and cross-reactivity in the unfractionated urine, shown in Figure 5.

The total amount of radioactivity in each of the five peaks A–E was determined, and expressed as a percentage of the total amount of radioactivity administered (45 μCi). The excretion of each of the five compounds over the first 24 hours after administration is shown in Figure 8.

It seems unlikely that glucuronide formation is a major route of LSD metabolism, since treatment of urine samples with either β-glucuronidase or sulphatase had no effect on the distribution of radioactivity in the radiochromatogram. In the present experiments it was not possible positively to identify the cross-reacting component in peaks A, B, C, and D. Mass spectrometry failed to show the presence of any LSD-like material in any of the peaks. The probable explanation of this was the extremely large (relative to the compound of interest) amount of other material present in the fractions. The only information obtained as to the identity of the peaks came from a consideration of their retention volumes on Spherisorb-5-ODS. Peak D had a retention volume (relative to LSD) of 0·36; this is similar to the value for lysergic acid monoethylamide and 2-oxo-LSD (which are not resolved under the conditions used); it is therefore possible that this peak contains one or both of these metabolites.

There are differences between these results and those obtained in the Rhesus monkey by Siddik (1975), whose work probably represents the most complete study of LSD metabolism to date. Siddik found that there were ten components in urine which were radioactively labelled when thin-layer chromatography was used as the separation technique. Of these, however, only five were tentatively identified by comparison of R_f values—the glucuronides of 13-hydroxy and 14-hydroxy LSD; lysergic acid monoethylamide; aromatised 2-oxo; and unchanged LSD. The major excretion product could not be identified—it did not correspond to any of the metabolites for which authentic samples were available, and although it was present in relatively large amounts no mass spectrum could be obtained. None of the metabolites found by Siddik could be identified as 2-oxo-LSD. The difference between the present results and those of Siddik are not altogether surprising, since different analytical techniques were used. In addition Siddik had samples of a greater number of authentic LSD metabolites and comparison with these allowed tentative identification of the radioactive urinary components.

It has therefore been shown that following oral administration of LSD to monkeys, the drug is rapidly absorbed from the gastrointestinal tract; the principal route of excretion is via the urine. Very little unchanged LSD could be detected in the urine after about two hours following administration. A similar situation has been found with forensic casework where urinary LSD concentrations were very low (Twitchett *et al.*, 1978). In addition, as in the

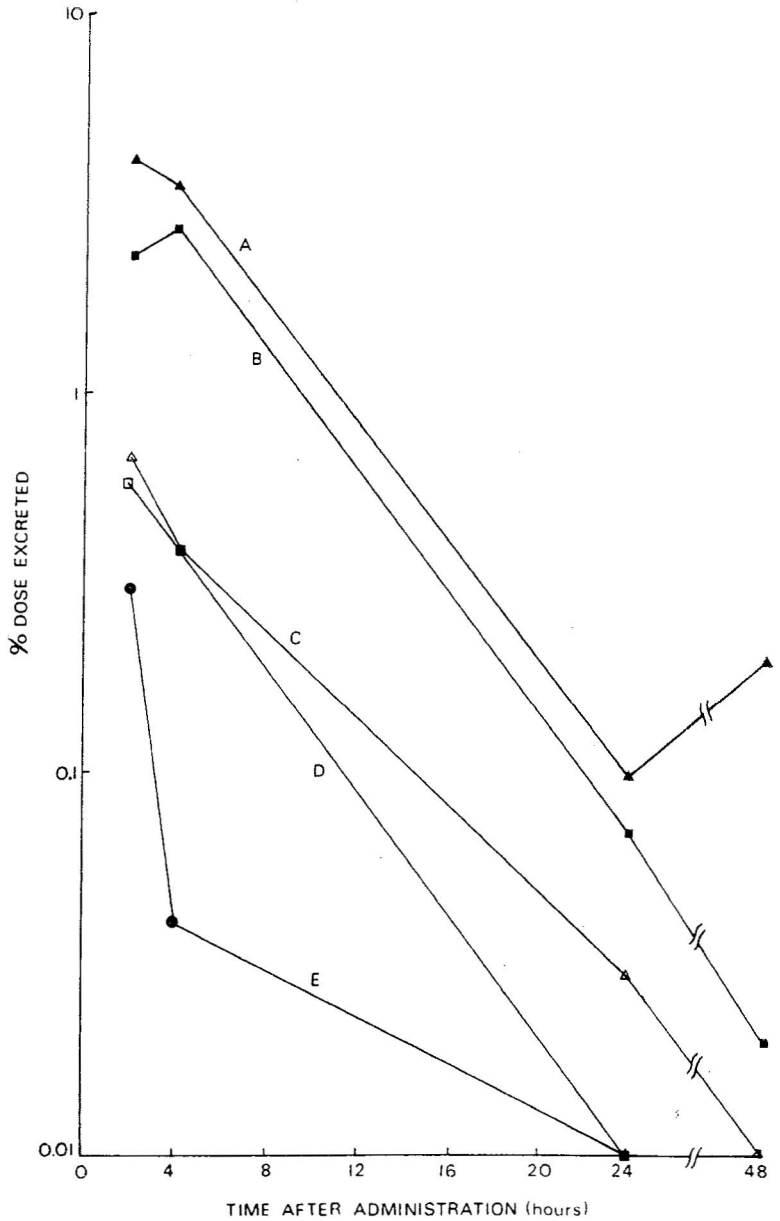

Figure 8. Urinary excretion of cross-reacting substances A–E following administration of ^{14}C-LSD (45μCi, monkey 171).

casework samples, other peaks of cross-reactivity were found in the *RIA* chromatograms of monkey urine. Since the substances found in these peaks were both labelled with ^{14}C and cross-reacted in the *RIA*, they must have been derived from LSD by metabolic processes in the body.

The results obtained in the monkey indicate that after oral ingestion of LSD, it may be possible to detect its metabolites in the urine for much longer than the parent drug and this situation should be similar in humans. The presence of LSD metabolites in a case sample further strengthens the evidence for prior

possession of the drug, and therefore is of great forensic significance. When the identities of the metabolites in humans have been firmly established, it should be possible to prove prior possession of LSD, even though the drug itself is no longer detectable in the body.

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