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THE DETERMINATION OF LSD IN HUMAN PLASMA FOLLOWING ORAL ADMINISTRATION

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SUMMARY

A spectrofluorimetric method is described for the assay of lysergic acid diethylamide (LSD) at nanogram levels in human plasma in the presence of a variable blank. The procedure makes use of the observation that ultra-violet light catalyses the hydration of LSD to the non-fluorescent lumi-derivative. The difference in fluorescence of plasma extracts before and after intense ultra-violet irradiation is a measure of LSD concentration.

This technique has been used for the assay of LSD in plasma following the oral administration of the drug at a dose of approximately 2 μg per kg body weight.

INTRODUCTION

Lysergic acid diethylamide (LSD; LSD 25) is pharmacologically active in man at doses as low as 1 μg per kg body weight. For its detection in body tissues a sensitive method is necessary and that of Axelrod *et al.*[1] is claimed to be specific and sensitive down to a level of 1 ng per ml of plasma. Aghajnian and Bing[2] used a modification of this method to measure plasma levels after intravenous administration of LSD and found a good correlation between plasma levels and performance test scores.

The present investigations were undertaken as part of a larger study of the psychological effects of LSD given orally. A single standard dose of 160 μg was given to healthy male volunteers and plasma was taken before and after administration. Preliminary observations indicated that the LSD levels would be below 10 ng per ml of plasma. It was also apparent that a plasma "blank" showed wide variation, not only between subjects but also in the individual subject. Moreover, the pre-dose blank was sometimes greater than the combined drug and blank levels in post-dose samples.

Repeated venepuncture was regarded as undesirable and therefore the volume of blood available for assay was limited. The problem, therefore, was to determine

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nanogram levels of LSD in small quantities of plasma in the presence of a variable blank.

MATERIALS

(a) LSD

Experiments were carried out with "Delysid" (Sandoz, Ltd) containing 100 μg of the base per ml.

(b) Blood samples

Venous blood was taken into glass bottles containing heparin ("Pularin", Evans Ltd). Samples were taken just before the drug was given and at intervals during the following five or six h. Plasma was immediately separated by centrifugation and stored at −20° until the following day when analyses were carried out.

(c) Reagents

(1) *n*-Heptane was redistilled and washed twice with *N* hydrochloric acid, *N* sodium hydroxide, and glass-distilled water. Iso-amyl alcohol was added to give a concentration of 2% (v/v).

(2) *N* sodium hydroxide and 0.004 *N* hydrochloric acid were prepared from concentrated volumetric solutions (C.V.S., British Drug Houses, Ltd) and stored in glass containers.

(d) Apparatus

(1) A Hanovia Ltd Laboratory Type Mercury Vapour Tube was used for preparative irradiation. Fluorescence was measured on an Aminco-Bowman Spectrophotofluorimeter.

(2) All glassware was washed in concentrated nitric acid and rinsed several times in glass distilled water. Contact of reagents with plastic containers was avoided since the latter were found to give rise to non-specific fluorescence.

METHODS

(a) Procedure of Axelrod et al.[1]

This consists of the extraction of the drug into *n*-heptane from plasma saturated with sodium chloride and made alkaline with sodium hydroxide, extraction from the heptane phase into 0.004 *N* hydrochloric acid, and measurement of the fluorescence of the acid extract.

(b) Modified procedure

The following description gives the detailed steps for measuring LSD in small volumes of plasma in the presence of a variable blank. Ultra-violet light catalyses the addition of a molecule of water across the C_9–C_{10} double bond of LSD to produce the non-fluorescent lumi-derivative[3].

(1) Extraction into heptane

Plasma 2 ml

Sodium chloride	1 g
Sodium hydroxide, *N*	0.1 ml
n-heptane	10 ml

These were rotated in 20 ml glass stoppered tubes on a Matburn cell suspension mixer at 27 rev/min for 30 min.

(2) Separation of the heptane extract

This was separated from the plasma and salt by centrifugation at 1500 *g* for 5 min.

(3) Extraction into acid

8 ml of the heptane extract was added to 3 ml of 0.004 *N* hydrochloric acid in a 20 ml glass stoppered tube. Extraction was effected by a combination of rotation (two periods of 5 min) and manual shaking for a few sec before, between, and after the periods of rotation.

(4) Spectrophotofluorimetry

The acid-heptane mixture was centrifuged to separate the phases, the heptane layer was removed by suction, and the acid extract was transferred to a 1 cm cuvette. The Aminco-Bowman Spectrophotofluorimeter was set to activation and emission wavelengths of 318 and 413 nm respectively. Fluorescence was measured on several gain settings, X 0.01 and X 0.003 being most suitable.

(5) Ultra-violet irradiation

After their fluorescence had been measured the acid extracts were transferred to glass tubes and exposed to ultra-violet light for 3 h. A semi-circle of tubes was arranged about 15 cm from a Hanovia mercury lamp with the filter removed. This lamp emits strongly at 253.6 nm. Minor peaks occur at 366, 404, 436 and 546 nm. The fluorescence was then read again. The decrease represented fluorescence of the drug and the residual fluorescence that was due to "blank" substances.

(6) Standards

LSD tartrate was added to plasma and extracted by the method given above. An acid solution, not subjected to the extraction procedure, was used as the "full recovery" standard. With 2 ml of plasma, 10 ml of heptane, and 3 ml of acid the drug concentration altered and the scale readings were multiplied by 1.88 to obtain the concentration in the starting material.

All procedures, from blood-taking to the reading of fluorescence, were carried out in dim light.

RESULTS

(A) Analytical observations

(a) Recovery of LSD added to plasma "in vitro"

Fig. 1 shows that fluorescence was linearly related to LSD concentration and that plasma samples yielded fluorescence values about 90% of those given by aqueous solutions similarly treated.

These results were obtained with the method of Axelrod *et al.*[1]. The modified method gave a similar relationship.

(b) Measurement of plasma blanks in volunteers

Preliminary studies with the Axelrod method indicated that the plasma blank before the drug was given was sometimes greater than the combined drug and blank level after administration.

Serial plasma samples obtained from men given a placebo (water) were extracted by the modified method and were shown to have a fluorescence equivalent to several nanograms of LSD. The pre-dose blank was sometimes much greater than that

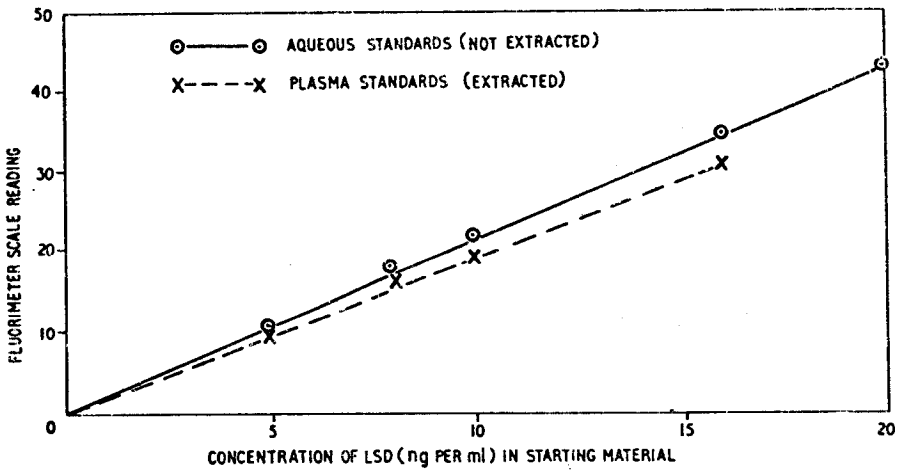

Fig. 1. Relationship between fluorescence and concentration of LSD in aqueous and plasma standards.

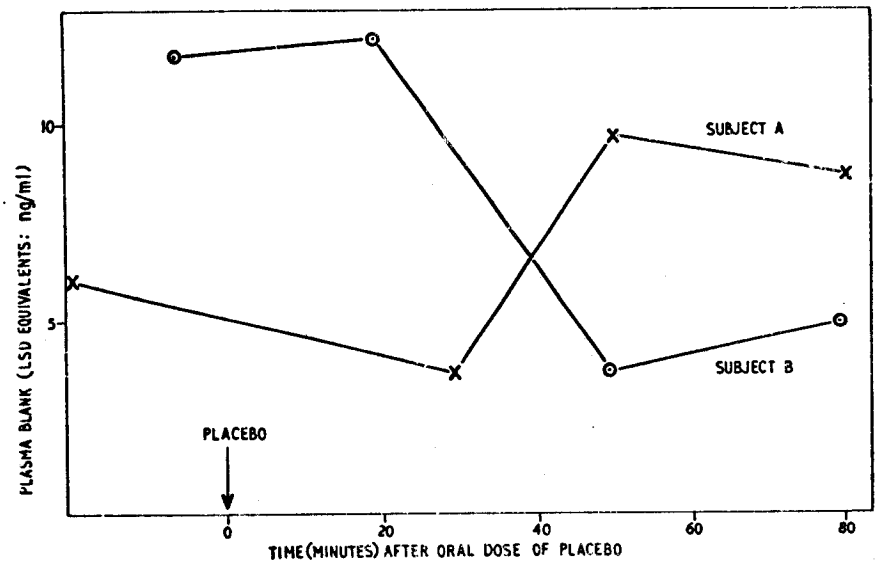

Fig. 2. Variation in plasma blank in two subjects receiving a placebo.

after dosing and was sufficiently high to obscure the expected plasma levels of the drug itself (Fig. 2).

(c) Measurement of the plasma blank and LSD

LSD was added in varying amounts to pooled plasma. The drug was extracted by the modified method and the acid extracts irradiated. The residual fluorescence in all samples after irradiation was the same, and the decrease in fluorescence was linearly related to drug concentration (Table I).

TABLE I

FLUORIMETER READINGS GIVEN BY PLASMA EXTRACTS BEFORE AND AFTER ULTRAVIOLET IRRADIATION

Concentration of LSD (ng/ml)	*Fluorimeter readings*		
	Before UV	*After UV*	*Difference*
0	0.078	0.081	0.003
6.8	0.216	0.075	0.141
13.6	0.380	0.074	0.306
20.4	0.540	0.073	0.468

TABLE II

LSD PLASMA LEVELS (ng/ml) IN 13 SUBJECTS

	Subject number	*Time (minutes) after oral dose of 160 µg of LSD*								
		20	*40*	*60*	*75*	*90*	*130*	*155*	*260*	*300*
Full meal	1	1.7	0.5	1.8	1.3	0.3	—	1.2	—	—
	2	1.5	2.8	3.7	3.2	1.8	—	3.0	—	—
	3	2.0	0.3	—	0.8	0.7	2.0	0	—	—
	Mean	1.73	1.20	2.75	1.77	0.93	2.0	1.40	—	—
No meal	4	5.8	4.8	5.6	3.7	—	—	3.2	—	2.3
	5	3.9	4.1	4.2	—	4.2	—	3.6	—	1.4
	Mean	4.85	4.45	4.90	3.7	4.2	—	3.40	—	1.85
Light meal	6	—	4.9	4.1	4.9	—	4.8	—	6.2	—
	7	1.5	2.5	4.9	6.9	—	8.8	—	—	2.9
	8	3.9	4.4	5.0	5.5	—	5.2	—	—	2.2
	9	3.4	5.5	2.8	3.3	—	1.1	—	1.7	—
	10	0.3	0.9	0.6	0.6	—	3.6	—	—	3.9
	11	3.3	4.5	3.3	3.8	4.6	—	2.8	—	—
	12	2.4	3.4	2.3	1.9	—	2.3	—	—	1.3
	13	2.1	3.0	2.5	7.6	—	3.3	—	2.7	—
	Mean	2.41	3.64	3.19	4.31	4.6	4.16	2.8	3.53	2.58
	SD	1.25	1.49	1.47	2.39	—	2.48	—	—	1.10

— = blood sample not taken.

(B) Application of the modified method to "in vivo" studies

(a) Recovery of LSD added to serial plasma samples

Venous blood was drawn from men given a light carbohydrate breakfast at approximately 30-min intervals. The plasma was removed and LSD was added to each sample to give a concentration of 10 ng per ml. Two series of seven samples were extracted, the acid extracts irradiated, and the decreases in fluorescence measured.

The mean recoveries of LSD were 82 and 85% with standard deviations of 8

and 7.5 respectively. At this level of LSD, therefore, the error was approximately ± 2 ng per ml.

(b) Measurement of LSD in plasma after oral administration

Three groups of volunteers were given an oral dose of 160 μg of LSD. Plasma was taken just before and at intervals in the following 5 h. Three subjects were allowed a full breakfast including fried food, two had none, and the remaining eight had a light meal of cereal, toast, butter, and tea.

The results summarised in Table II were obtained with the modified procedure. Because of clinical and psychological examinations it was not possible to take blood at exactly the same intervals, and in Table II observations made within 15 min of the times listed have been grouped together.

There appeared to be a difference in LSD levels between men who had eaten a full breakfast and those who had not eaten. Men given a light carbohydrate meal had plasma levels in between these extremes. Although the number of subjects in each series was small and did not allow close statistical analysis, the results suggest that the ingestion of food and its composition had an effect on plasma LSD levels, probably because of the difference in rates of intestinal absorption.

The mean plasma levels of the three groups are given in Table II. LSD was detectable for up to five hours in those men who had fasted or had eaten a light meal. Plasma samples were not taken later than some $2^1/_2$ h after dosing from those men who had eaten a full meal, but it is unlikely that the drug would have been present in sufficient concentration for its accurate estimation at 5 h.

DISCUSSION

LSD is a highly active drug, its effects being apparent after oral administration of doses in excess of 1 μg per kg. Such doses result in nanogram levels per ml of plasma. The object of the present study was to develop an analytical method capable of measuring such small amounts accurately.

Aghajnian and Bing[2] measured plasma levels of intravenously administered LSD and noted the presence of a blank unless the apparatus had been specially cleaned. We took precautions and were able to avoid non-specific fluorescence from contaminants. A blank fluorescence was observed, however, and it was of sufficient magnitude seriously to interfere with the determination of LSD. This interference was eliminated by applying the observation of Stoll and Schlientz[3] that ultra-violet irradiation of LSD results in a non-fluorescent compound.

The activation and emission spectra of this blank were broad and had ill-defined maxima close to those of LSD. We did not investigate the chemical nature of the blank, and we are unable to say whether it represented a substance present in nanogram amounts in plasma or one present in higher concentration and extracted in part with the LSD fraction.

The possible delaying effect of a meal on LSD absorption was illustrated by the lower plasma levels observed in men who had eaten a full breakfast. The composition of a meal, the pH of gastric and intestinal contents, and the rate of gastric emptying markedly influence the absorption of a drug, and standardisation of the meal in eight subjects appeared to reduce some of the variation in plasma levels.

The conditions of ingestion of illicitly obtained LSD will obviously vary very

widely. The dose, however, could be greatly in excess than that used in the experiments described here. Psychotic patients often receive doses at least twice as great as that given to our subjects. It would appear, therefore, that the method we describe would be of value in detecting and accurately measuring LSD some hours after ingestion, whether or not a meal had been taken, in patients or in persons taking the drug illicitly.

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