URINARY CANNABINOID EXCRETION PATTERNS

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I. INTRODUCTION

Investigators have shown that the rate of excretion of the many urinary metabolites and cannabinoid constituents following active smoking of marijuana is variable from individual to individual as well as for a single individual over time. Although the peak psychoactive and physiologic effects appear within 2-3 minutes and can last from 90 to 120 minutes after smoking a single cigarette, elevated excretion of urinary metabolites occurs within hours after exposure and levels have been shown to remain detectable for many days (1-11).

Since the variability in excretion of metabolites coupled with variabilities in fluid intake, frequency of urination and differences in kidney function exist, correlation of urine values with intoxication or time since use may not be feasible. It has been reported, however, that delta-9-tetrahydrocannabinol (THC) is more rapidly metabolized in chronic users than naive users and that frequent users often exhibit basal urinary values that exceed peak values attained by infrequent users (12-14).

The present study, therefore, was designed to monitor daily urine samples from several subjects following an alternating smoking/abstension protocol over a 25 day period. Comparison of the sample results to the dosing schedule was done in order to determine: the duration of positive results, the presence of observable trends in metabolite excretion and the existence of differences in excretion patterns for frequent vs. less frequent users.

II. MATERIALS AND METHODS

The clinical aspects of this study were conducted at the University of North Carolina, Department of Psychiatry, by Dr. Mario Perez-Reyes. The subjects involved were selected volunteers from a group well known to Dr. Perez-Reyes and his staff and who had been found reliable in previous marijuana clinical research.

Three male and three female subjects, all experienced marijuana users, were asked to refrain from smoking for seven days prior to the beginning of the investigation. Subjects DF_2 and DM_3 stated that they were frequent users (~ daily). Subjects DF_1, DF_3, DM_1 and DM_2 were less frequent users (1-4 times/week). All subjects in this study also provided blood samples for a concurrent study conducted by Dr. Mario Perez-Reyes (15). After 7 days of abstinence from marijuana use, each subject provided a baseline urine sample. Using a randomized, double-blind sequence, subjects were required to smoke one marijuana cigarette each week for three weeks. Standardized cigarettes were obtained from the National Institute on Drug Abuse (NIDA) and contained 1.32%, 1.97% or 2.54% of THC. The subjects were required to refrain from further marijuana use for the duration of the study and for 10 days following the last cigarette. Urine samples were collected daily for the duration of the smoking study and for 10 days after smoking the third cigarette.

Subjects were instructed to provide the samples from the first urination each morning. On the days that a cigarette was smoked, the urine sample was collected prior to smoking in

TABLE I. Multiple Dose Study: Dosing Sequence

Subject	Stated frequency of prior use	Dosing Sequence (%THC/cigarette)		
		Day 1	Day 8	Day 15
Males:				
DM1	1-4x/week	2.54	1.97	1.32
DM2	1-4x/week	1.32	2.54	1.97
DM3	~ daily	1.97	1.32	2.54
Females:				
DF1	1-4x/week	2.54	1.32	1.97
DF2	~ daily	1.32	1.97	2.54
DF3	1-4x/week	1.97	2.54	1.32

TABLE II. Performance Characteristics: EMIT-d.a.u. and EMIT-st Urine Cannabinoid Assays

Parameter	EMIT-d.a.u. Semiquantitative Assay	EMIT-st Qualitative Assay
Cutoff Calibrator Concentration	20 ng/ml 8-THC Acid*	100 ng/ml 8-THC-Acid*
Sensitivity	ca. 20 ng/ml Cannabinoids	ca. 100 ng/ml Cannabinoids
Minimal concentration detected as Positive with 95% confidence	50 ng/ml Cannabinoids	200 ng/ml Cannabinoids
Confidence limit at 0 ng/ml for Negative response	at least 95% confidence	at least 95% confidence
Specificity:	Class Specific for Cannabinoids: delta-9-THC 11-Nor-delta-9-THC-9-carboxylic Acid 11-Hydroxy-delta-9-THC 8-beta-Hydroxy-delta-9-THC 8-beta,11-Dihydroxy-delta-9-THC	

*Performance of the 11-nor-delta-8-THC-9-carboxylic acid (8-THC Acid) employed in the calibrators has been shown to produce a response essentially equivalent to that of the 11-nor-delta-9-THC-9-carboxylic acid metabolite.

the morning; the samples collected on days 2, 9 and 16 were the first samples collected following smoking.

The individual dose sequence for each subject is given in Table I.

A. Analytical Method

Analytical determinations on the urine samples were conducted by the Clinical Studies Laboratory, Syva Company. All clinical samples were sent blind and included control specimens. The sample code was supplied following analysis.

The EMIT-st and EMIT-d.a.u. Cannabinoid Assays employ a homogeneous enzyme immunoassay technique for the microanalysis

of specific compounds in biological fluids (16-18). A drug is labeled with an enzyme, and when the enzyme-labeled drug becomes bound to an antibody against the drug, the enzyme's activity is reduced. Drug in a sample competes with the enzyme-labeled drug for the antibody binding sites, thereby decreasing the antibody-induced inactivation of the enzyme. Active enzyme converts NAD^+ to NADH, resulting in an absorbance change that is measured photometrically (EMIT-st) or spectrophotometrically (EMIT-d.a.u.). The enzyme activity in the sample mixture is compared against the activity of the Cutoff calibrator in order to interpret the result. Samples producing a response greater than the Cutoff calibrator response are interpreted as Positive; those producing a response less than the Cutoff calibrator response are interpreted as Negative.

The EMIT-d.a.u. Cannabinoid assay is a semiquantitative technique employing a Negative (0 ng/ml), a Cutoff (20 ng/ml) and a Medium (75 ng/ml) calibrator. The Emit-st Cannabinoid assay is a qualitative technique employing one Cutoff calibrator (100 ng/ml). The performance characteristics of the assays are given in Table II.

The methods detect the presence of THC by assay of urinary cannabinoid constituents and metabolites. Both assays are most sensitive to 11-nor-delta-9-THC-9-carboxylic acid and to 11-hydroxy-delta-9-THC (12,14). Since it has been shown that 12 hours following the administration of THC, less than half of the total 11-nor-delta-9-THC-9-carboxylic acid is present in unconjugated form, the assays are also designed to be sensitive to both conjugated (glucuronide) and unconjugated forms of urinary metabolites (19).

III. RESULTS

A. EMIT-d.a.u. Analysis

All samples were frozen on the day of collection and thawed on the day of analysis. All the samples from each subject were assayed as a batch and were, thus, compared to the same Low Calibrator response for interpretation. Samples and calibrators were analyzed in duplicate; the results are expressed as the average of two determinations.

The daily sample responses for each subject are plotted in Figure 1. In order to compare intersubject variability, the results are presented as the differences obtained between the Low Calibrator response and the sample response.

As can be seen in Figure 1, the excretion pattern for each individual is different; however, general trends can be observed. All subjects produced a Positive response the day following smoking. And, in most cases, each of the three peaks occurred the day following smoking and each peak response was followed by responses that exhibited a declining trend over time. In several cases, it was noted that as the daily responses approached that of the Cutoff calibrator, the results vascillated between Positive and Negative interpretations. This effect is expected when excretion of cannabinoids decreases to the sensitivity level of the assay (20 ng/ml) and can be due primarily to normal variations in the urine.

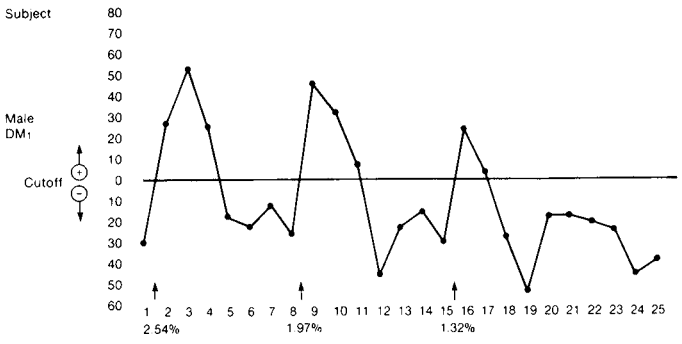
Of the subjects who admitted to less frequent prior use (DF_1, DF_3, DM_1 and DM_2), all produced a pre-dosing Negative specimen on Day 1. Following smoking episodes, each subject generally produced 2-5 consecutive Positive samples followed by declining values until the last dose. As can be seen from the Figure, although some variability is apparent, peaks corresponding to the doses smoked which are followed by declining trends during the abstension periods are observable.

For the frequent prior-users (DF_2 and DM_3), after six days of abstinence from marijuana use, the pre-smoking (Day 1) samples produced Positive results; the sample from subject DF_2 exceeded the response of the 75 ng/ml Calibrator. With the exception of three samples obtained during the third week of the study, these subjects also remained consistently Positive for the duration of this investigation. In fact, positive samples were still being obtained at the end of the study, which was 10 days following the final smoking episode. These results illustrate the results of previous studies showing that chronic and/or high doses of marijuana can produce an accumulation of cannabinoid constituents in the body that are detectable in the urine for 10 days or longer with this analytical method (20,21).

Samples from subjects DF_2 and DM_3 are representative of chronic and/or high dose marijuana use. These subjects consistently produced higher responses than samples from other subjects and also produced the greatest number of consecutive Positive responses of the subjects studied. These analytical findings are consistent with the known marijuana smoking habits of the subjects.

For all subjects, following the peak occurring after the third dose, the responses obtained gradually declined; by Day 25, all subjects had produced at least one sample which was designated as negative.

No correlation between the % THC in the dose administered and the responses obtained was calculated.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Male
DM1
Cutoff
+
-
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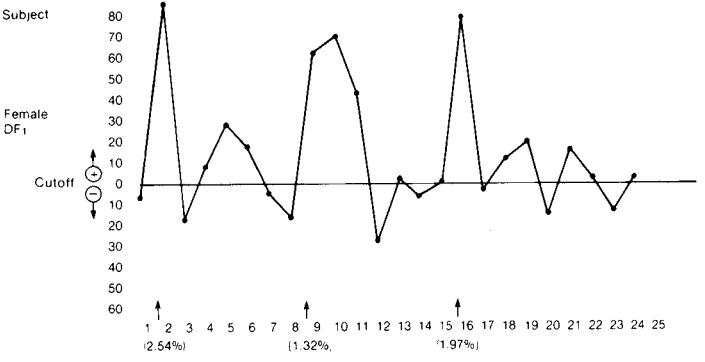
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
2.54%
1.97%
1.32%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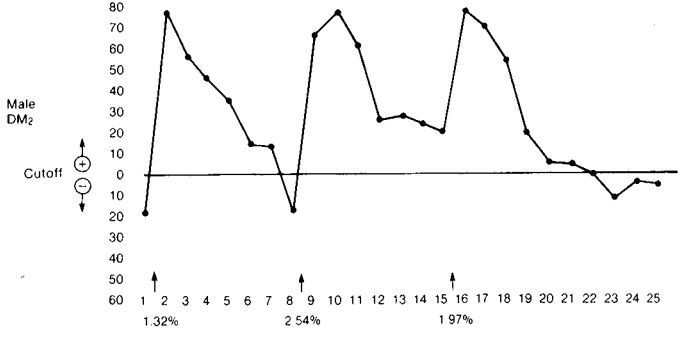
Female
DF1
Cutoff
+
-
80 70 60 50 40 30 20 10 0 10 20 30 40 50 60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
(2.54%)
(1.32%,
(1.97%)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DM2
Cutoff
+
-
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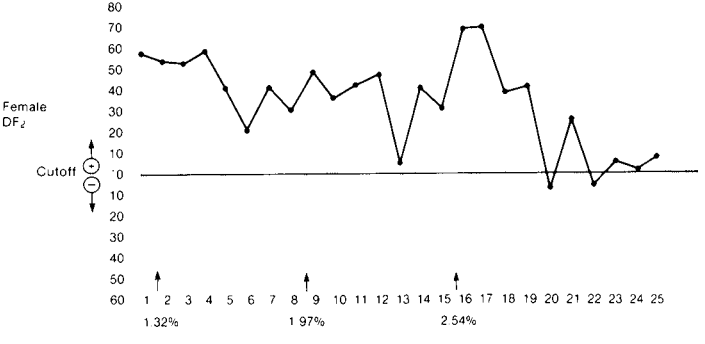
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
1.32%
2.54%
1.97%


Female
DF2
Cutoff
+
-
80 70 60 50 40 30 20 10 0 10 20 30 40 50 60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
1.32%
1.97%
2.54%

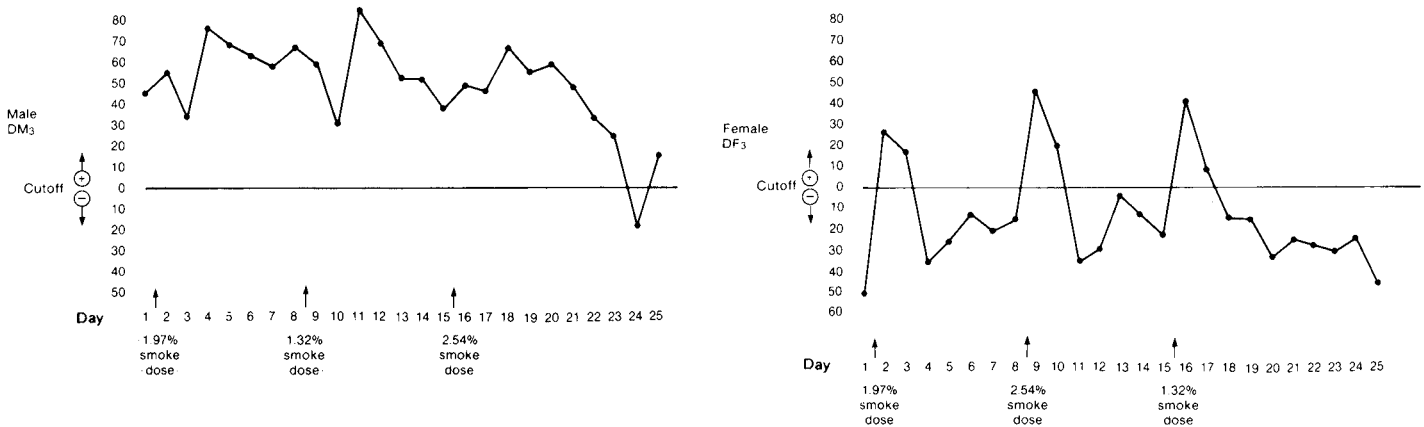


FIGURE 1. Males and Females: Individual Values vs. Time (Days). Samples response is expressed as the of the low Calibrator - of the sample; Cutoff response is expressed as "0".

B. Comparison of Daily Total Positive Values (EMIT-d.a.u.)

The data in Figure 1 was combined (Figure 2) and the percent of Positive samples was calculated for each day. From the figure it can be seen that 100% of the subjects produced positive samples one day after smoking which declined to approximately 50% 6 days after smoking and to 30% after 9 to 10 days.

C. Analysis of Samples by EMIT-st and Comparison Results with EMIT-d.a.u. Results

In order to illustrate the detectability of cannabinoids as a function of assay sensitivity, all samples were subsequently analyzed using the EMIT-st qualitative assay. As can be seen in Figure 3, since the EMIT-st assay has a 100 ng/ml sensitivity vs. 20 ng/ml for the EMIT-d.a.u. assay, interpretation of sample responses using the ST cutoff resulted in significantly fewer total Positive results. In general, however, following each smoking episode, excretion of cannabinoids was sufficient to produce at least one sample that was analyzed as positive.

From the Figure 1, it can be seen that the less frequent users produced no more than a total of 3 positive EMIT-st responses following each dose. These subjects also produced consecutively negative results for the final 9 days of the study. Of note was subject DM_1 who, although he smoked the three weekly doses, produced responses less than that of the 100 ng/ml calibrator for the duration of the study.

In contrast, the frequent smokers (DF_2 and D_3)excreted cannabinoids at levels consistently detectable by the 20 ng/ml D.A.U. cutoff as well as the 100 ng/ml ST cutoff for the first two weeks of the study. Following the final dose, subject DM_3 excreted sufficient cannabinoids to produce 7 consecutive responses by both assays. And, on the 10th day following the final dose, this subject also produced positive responses by both assays. Although samples from subject DF_2 produced responses greater than D.A.U. 20 ng/ml calibrator for 8 of the final 10 days of the study, only the first 4 samples collected after smoking contained sufficient cannabinoids to also produce responses greater than the 100 ng/ml ST cutoff. Again, the variability seen from subject to subject can be attributed to differences in excretion rates as well as to normal variabilities in urine; the excretion patterns observed, however, appeared to be related to the subject's prior frequency of use as well as to the doses smoked.

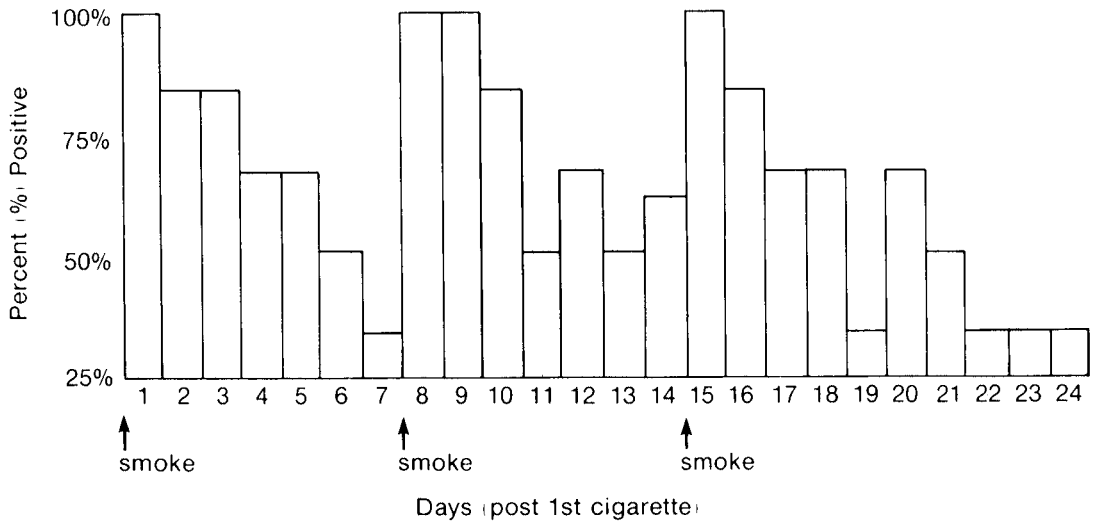
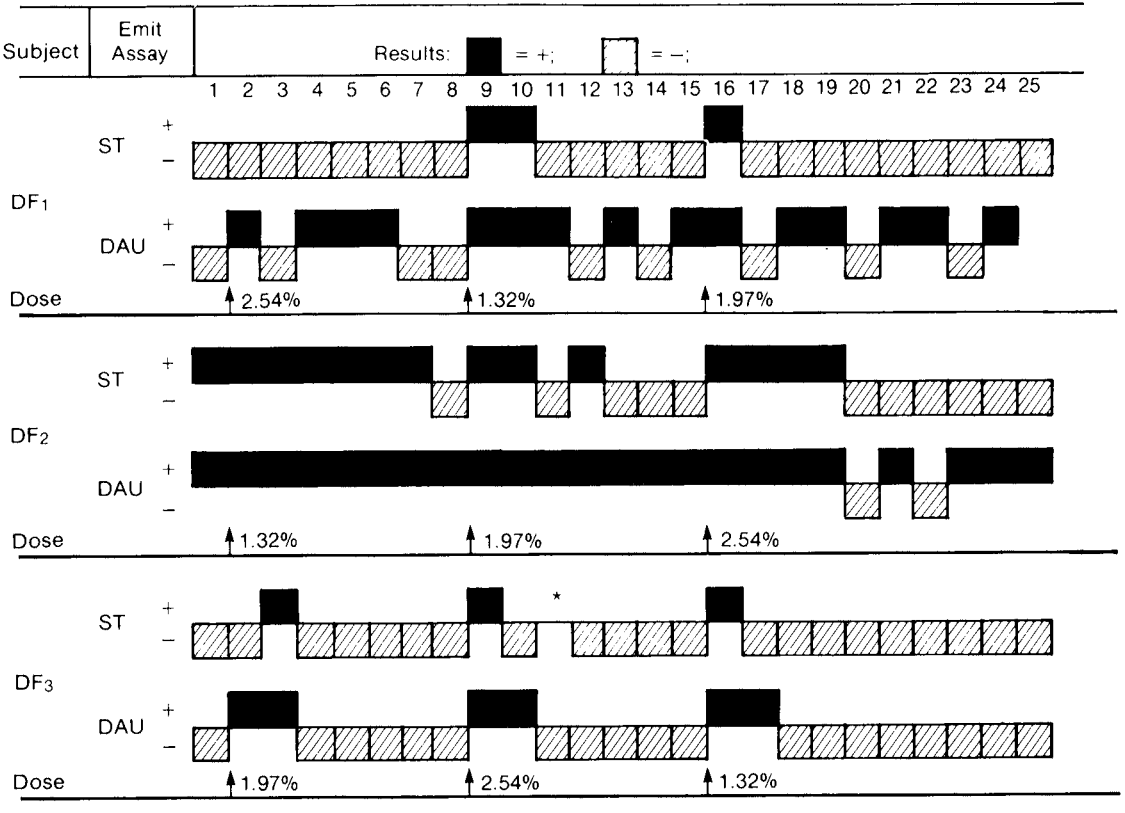


FIGURE 2. Percent of Samples Producing Positive Responses
(First Urine Post-Smoking - 10 Days Following Final Cigarette)


Subject
Emit Assay
Results: = +; = –;
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
ST
DF1
DAU
Dose
2.54%
1.32%
1.97%
ST
DF2
DAU
Dose
1.32%
1.97%
2.54%
ST
DF3
DAU
Dose
1.97%
2.54%
1.32%

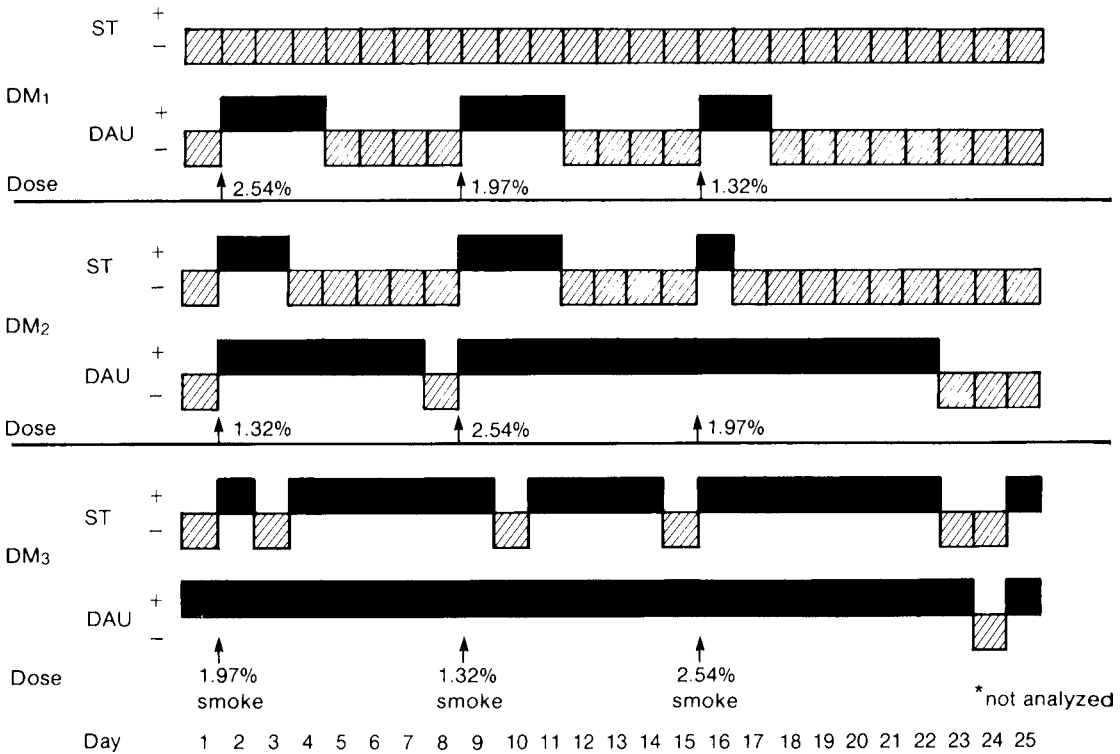


FIGURE 3. EMIT-st and EMIT-d.a.u. Comparison of Results for the Same Samples

D. Creatinine Study

Since the volume of urine produced can have an effect on the apparent concentration of a given substance, ng THC metabolite per mg creatinine was calculated for each sample. The results were then compared day to day and to each smoking episode in order to normalize the trends observed by analysis of the EMIT-d.a.u. data for possible dilution effects.

As can be seen from Figure 4, the ng THC metabolite per mg creatinine excretion patterns exhibit three distinct peaks that occur following smoking. Over time, the responses obtained declined between cigarettes and after the last cigarette until the end of the study. Although variability was apparent, the results exhibited patterns that appeared to be similar to those observed from the EMIT results. And, consistent with the EMIT data, the more frequent users (DF_2 and DM_3) were found to excrete cannabinoids at higher levels for greater numbers of consecutive days than the less frequent users.

E. Results Comparison

The results of these studies are consistent with the results of two previously reported smoking studies (20) also conducted by Dr. Perez-Reyes. In those studies, a total of 3 males and 4 females (all experienced users of marijuana) were asked to abstain from use for 6 days, to then smoke two 9 mg THC cigarettes within two hours and to abstain from further use for 10 days. Urine collected daily for 10 days and was stored and analyzed using the EMIT-d.a.u. assay. By comparing the total number of Positive results obtained each day following dosing (Figure 5), the trend was very similar to that observed following the third cigarette in the present study. Initially all samples produced Positive results; 45% of the samples were positive after 5 days and 25% after 10 days.

IV. DISCUSSION

A. EMIT-d.a.u.

Significant individual and subject to subject variability was observed; however, three peak responses corresponding to the smoking episodes were observed. In all cases, samples collected the day following smoking contained sufficient metabolites to produce Positive results. For the seventh through the tenth day of abstention, no more than 30% of the

samples collected produced Positive results.

In general, the highest responses observed were those on the day following smoking; the responses then showed a general tendency to decline until the next dose. By the fourth day following the final cigarette, the responses obtained from all subjects showed a tendency to decline; no responses equal to or greater than the peak following the third cigarette were observed for the remainder of the study.

Of the less frequent users, all produced pre-smoking, Day 1 samples that were interpreted as Negative. Generally these subjects were found positive 2-5 days after each dose. In contrast, frequent users produced pre-smoking Positive responses and remained Positive for almost the duration of the 25 day study. Thus, the excretion patterns for both groups were dissimilar and the duration of detectable cannabinoid excretion was greater for the frequent users.

B. EMIT-st

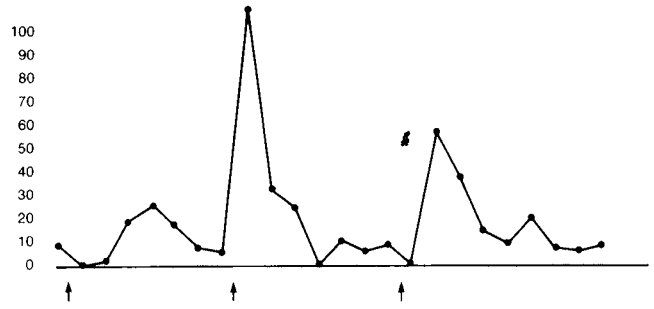
Of the subjects who began the study with pre-smoking Negative results, all produced Positive responses for 1-3 days. These subjects also produced Negative results for the final nine days of abstinence following the third cigarette.

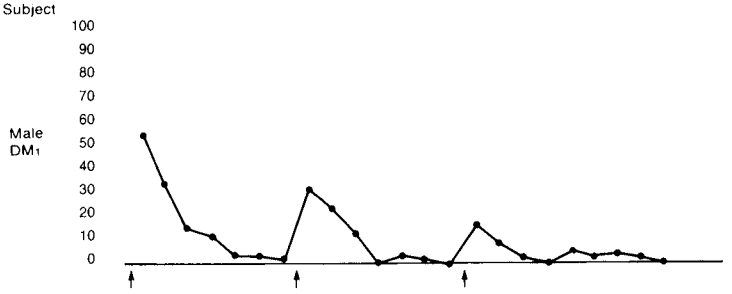
The two frequent users (DM_3 and DF_2) produced a greater number of consecutive daily Positive responses following each dose; both subjects produced as many as seven consecutive daily responses following one cigarette. By the tenth day following the final dose, subject DM_3 excreted sufficient metabolites to produce a Positive response; all other subjects had produced at least 6 consecutive daily Negative responses.

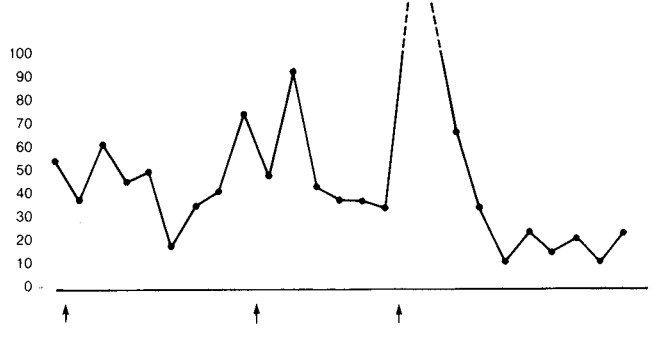
The trends observed were consistent with previous studies, and were confirmed when the data from both EMIT-st and EMIT-d.a.u. determinations were analyzed relative to creatinine excretion.

V. SUMMARY

Although general patterns were observed in the results obtained, the results are not indicative of intoxication or the time elapsed since using marijuana. In general, however, the difference in the duration of consecutive Positive responses for less frequent users versus frequent users appeared to be the result of cannabinoid metabolite excretion due not only to the doses administered but to the subject's previous habit of frequent use.


100
90
80
70
60
50
40
30
20
10
0


Subject
Male
DM1
100
90
80
70
60
50
40
30
20
10
0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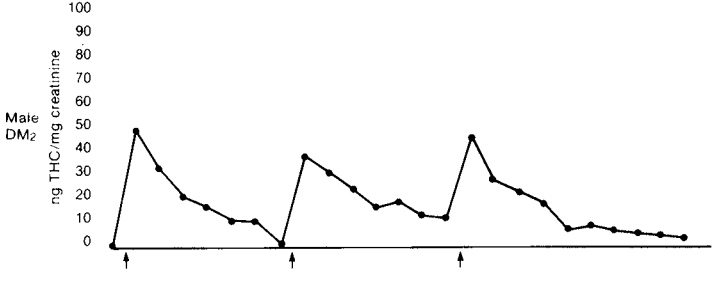
Male
DM2
ng THC/mg creatinine
100
90
80
70
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20
10
0

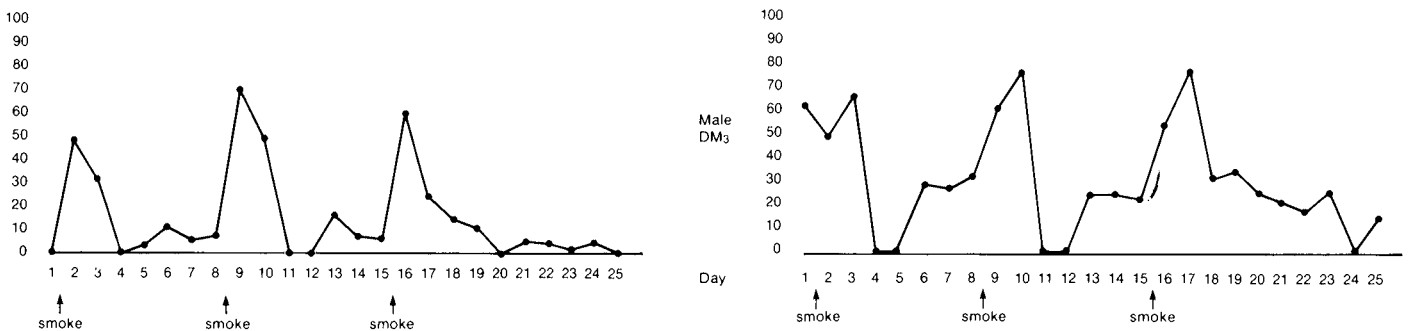

FIGURE 4. Males and Females - ng THC metabolite per mg creatinine per day.

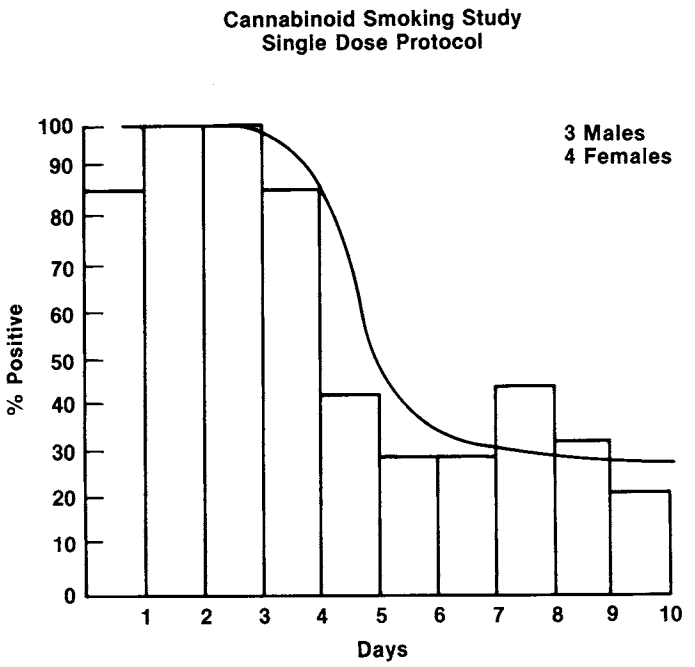

FIGURE 5. Percent of EMIT-d.a.u. Positive responses obtained per day following smoking of two marijuana cigarettes (n = 7).

EMIT-d.a.u. urinary excretion patterns, following weekly smoking episodes in a three week period, exhibited cyclic peak and decline profiles which allowed differentiation of each smoking episode. Peak response occurred 1-2 days after smoking and less frequent users remained Positive for 2-5 days while frequent users remained Positive for up to 10 days. A general declining trend followed the peak level, for all subjects.

Excretion patterns were highly individual, however cannabinoids were also detectable by the EMIT-st 100 ng/ml calibrator for up to 10 days following smoking for frequent users while less frequent users produced no more than 3 EMIT-st Positives following any dose.

Data on blood levels of THC, psychological rating and heart rate acceleration of these subjects were also collected according to a double blind protocol. All three parameters could be correlated with dose of marijuana (15).

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