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Plutonium in Human Urine: Normal Levels in the U.S. Public

Volume II

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Plutonium in Human Urine: Normal Levels in the U.S. Public

(Cooperative Agreement DE-FC08-89NV10865)

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FISSION TRACK ANALYSIS OF PLUTONIUM IN HUMAN URINE SAMPLES

Interim Report to the DOE

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An Ultrasensitive Technique for Measuring Plutonium in Human Urine Samples by Fission Track Analysis

ABSTRACT

A neutron induced fission track method was successfully developed for assaying ^{239}Pu in human urine with a detection limit below 20 aCi/sample. The technique involves the co-precipitation of ^{239}Pu with rhodizonic acid, separation of ^{239}Pu from potentially interfering natural uranium and other inorganic materials by ion-exchange techniques, collection of the sample onto lexan detectors, irradiation of sample in MIT reactor at a fluence of 1.1 x $10^{17} n/cm^2$, etching of the lexan slide and counting the track either manually or by some automated counting system.

Introduction:

The most commonly employed technique for measuring plutonium in biological and environmental samples is alpha-spectrometry. Unfortunately, the lower limit of detection by alpha-spectrometry (0.01 pCi/sample) is quite high (~three orders of magnitude) than needed to measure the urinary excretion of ^{239}Pu in general population who acquired about 3 pCi of $^{239,240}Pu$ from inhaling the dust contaminated with plutonium from global fallout of open air nuclear testings in the 1950s.

Mass-spectrometry has not been utilized to measure plutonium at this low level either. We, therefore, developed fission track assay for measuring ^{239}Pu in human urine to establish the daily excretion of background level of ^{239}Pu in general population. The basic aspect of this technique is described briefly. Fissionable materials such ^{233}U, ^{235}U and ^{239}Pu undergo thermal neutron induced fission and two fragments per fission are emitted in opposite directions. One fragment per fission produced from a source in which all of the fissile atoms are at the interface between the source and the detector will produce a detectable track. Therefore, measuring the number of fission tracks produced in a sample collected on a lexan detector quantifies the activity of ^{239}Pu.

Radiochemical Procedure

Collection and Preservation of Samples:

The subjects are provided with a sample collection kit which contains two 2 liter polyethylene bottles, a pair of gloves, packs of blue ice and instruction sheets. The subjects are asked to collect 48 hours urine samples in the bottles provided and preserved in the kit with blue ice. Upon the collection of samples, they bring the samples to the laboratory which are acidified with HCl to pH1. If the samples are not processed immediately, they are frozen in the freezers.

Co-precipitation of Pu from Urine Samples:

The urine samples collected in the bottles are thawed and shaken very well to ensure proper mixing. The desired amount of the sample is poured into a proper size Erlenmeyer flask. Sodium hydroxide solution is added to the urine sample to adjust the pH to 2. Appropriate amounts of rhodizonic acid is added into the urine sample with constant stirring until the acid dissolves completely. A solution of NaOH is added to adjust the pH to 9.5. A dark brown precipitate forms, which is allowed to settle for 10 minutes. Absolute ethanol is added until the volume is doubled.

The precipitate is separated by centrifugation, dried, and dissolved in small portion of HCl. The solution is evaporated to dryness, digested with HNO_3 and reevaporated to dryness. Iron content of this solution is removed by dissolving the residue in 10 M HCl and extracting with isopropyl ether.

Ion Exchange Separation of Pu:

Plutonium is separated from the bulk of inorganic materials present in the urine by the ion-exchange technique. The solution obtained after the removal of iron is prepared in 8NHCl and passed through a column containing an ion exchange resin (a mixture of 100-200 mesh and 200-400 mesh, AG-1 X8) conditioned with 8N HCl and 8N HCl + Cl_2 mixture. The column is washed with 8N HCl. Plutonium is eluted with 8N HCl + HI and collected in a quartz crucible. The solution is evaporated to dryness, digested with small amounts of clean HNO_3 and finally by 50-100 ml of 8N HCl. The solution is evaporated to dryness and redissolved in very small amounts of 8N HCl.

The solution thus obtained is passed through another column to repeat the above procedure except that the column size is much smaller than the first column. The resins used are a mixture of various mesh sizes AG 1 X 8. The solution containing plutonium is passed through the above column conditioned with 8N HCl and finally with 8N HCl + Cl_2. The column is washed with 8N HCl and plutonium is eluted with 8N HCl +HI and collected directly onto the lexan slide. The solution collected as drops is dried by slow evaporation process. The slide is stored in the desiccator until all the slides are ready for packing in the rabbit.

Packing of the Slides:

The slides are stacked on each other in the following manner: A blank slide is placed on the bottom. The slide containing the sample (sample side up) covered with a piece of Makrofol, is kept on the top of the bottom slide, followed by all other slides containing the samples in the same manner. On the top of all the slides, another blank slide is kept. The whole stack of slides are taped together on one side so that they are held together firmly. The stack is placed in the rabbit using enough spacers so that the stack fits properly and remains firm inside the rabbit. The rabbit is then shipped to MIT Reactor for irradiation at a fluence of 1.1×10^{17} n/cm^2.

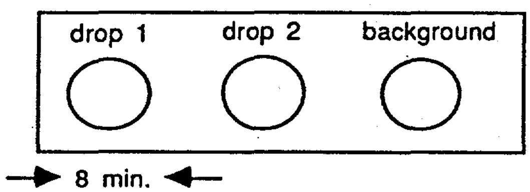
Etching of the Samples:

After receiving the rabbits back from MIT (after irradiation), a radiation survey of the rabbit and the shipping container is performed and recorded. Once the radiation level is less than 5 mR/hr, the rabbit can be unpacked with proper precautions, such as cleaning and identifying the area of work, wearing surgical gloves, etc. The bottom piece of lexan is removed carefully making sure that the piece of Makrofol on the top of it is not torn or repositioned. Using the "sharpie" marking pen, the Makrofol is coded with the same code as the lexan slide covered with the Makrofol. The process is repeated until all the lexan slides are uncovered from the Makrofol and all the Makrofols are coded appropriately. The lexan slides are transferred onto a paper towel.

Six lexan slides attached to a teflon stirrer are dropped into a solution of 6.5 N KOH, heated in a water bath at a temperature of 70°C. They are constantly rotated at a very slow speed in KOH solutions for about 11 minutes. After 11 minutes have elapsed, the slides are removed from KOH solution and transferred to a beaker containing deionized water and stirred for 5 minutes. This process rinses the slides very well after which they are briefly dropped into ethyl alcohol. The lexan slides are removed from the alcohol and placed on paper towels and air dried. The process is repeated until all the slides are etched as described above. Finally, the lexans are placed onto clean glass slides and taped into place.

Track Counting

Tracks formed are counted either manually or by semi-automated counting system. The sample area is scanned line by line either horizontally or vertically, whichever is more comfortable to the person counting the tracks. The tracks look like carrot shaped holes in the lexan between 5 and 20 microns long (Figure 1). The net track count for a sample is equal to the sum of the tracks in the two elution drops less twice the track count in the background area for that slide as given below:



Lexan slide with two drops of sample and one drop as background.

Reagent Blanks:

It is imperative to measure the number of tracks in the reagents utilized in radiochemical process of the determination ^{239}Pu. Therefore, the amount of rhodizonic acid (equivalent to the amount used in actual samples) is dissolved in 8N HCl as if rhodizonic acid contains plutonium. The 8N HCl solution thus obtained is processed through the entire procedure as described earlier. The number of tracks thus obtained due to reagents is subtracted from the total number of tracks obtained in a urine sample to give the net tracks due to plutonium in a human urine sample.

Yield and Track Registration Efficiency:

The net effect of track registration efficiency and yield was determined in two different ways. First, the measurement of the radiochemical yield from the first column (determined by gross a measurements from 8 radiochemical tracer experiments in which ^{239}Pu and ^{242}Pu were added to urine)(82 ± 16%), was multiplied by the combined yield and track registration efficiency (2.15 tracks/aCi)(determined when plutonium was eluted onto Lexan through the second column). This resulted in an expected yield and registration efficiency of 1.76 tracks/aCi. The second method was by direct radiochemical yield determination from three spiked urine samples, which gave a net yield and registration efficiency of 1.42 tracks/aCi with a range between 1.12 and 1.67 tracks/aCi. We took the mean of the two as our best estimate of yield and registration efficiency which equals 1.60 tracks/aCi of ^{239}Pu. The results are given in Table 1.

Results and Discussions

Results for Control Urines:

Acceptable analyses were obtained in 26 of 28 control urine analyzed in various rabbits (rabbits 26 through 32). The means and standard deviations of net tracks in 26 control urine samples (or subgroups of the 26) taken from 4 young volunteers born on or after 1963 are shown in Table 2. Table 3 shows the details of results in 28 analyses of controls, of which 2 were rejected as statistical outliers. Our hypothesis is that such young people have very little ^{239}Pu in their bodies (and consequently little in urine) since they were born after most deposition of global ^{239}Pu had already occurred and consequently they could not have accumulated much by direct inhalation. It is assumed that accumulation from inhalation of resuspended dust and ingestion in food is also negligible. Although these assumptions appear reasonable, we are not certain they are correct.

The mean number of tracks found in the control urines was 40 tracks with a standard deviation of 14 tracks. We have tested the results on these 26 controls from 5 irradiation for normality using the Kolmorogov-Smirnov test for normality and normality was not rejected (p=0.23).

TABLE 1

Yield of Pu from Urine, 1st Column
(Tracer Pu-238,242)

86.0

97.8

99.7

74.1

87.1

78.4

54.0

---

mean = 82.4% ± 16 (1 s)
yield and registration efficiency
(column 2 only) = 2.15 tracks/aCi
product = 1.77 tracks/aCi

Track Yield (tracks/aCi) from Urines Spiked with 250 aCi Pu-239

1.67

1.12

1.46

mean = 1.42 tracks/aCi

The mean of the two techniques is (1.77 ± 1.42) = 1.60 tracks/aCi

TABLE 2

Control Urines** (Net tracks)

# of irradiation	n	mean±1s
R24	none	
R26	10*	49±11
R28	6	35±11
R29	4	45±14
R30	4	33±13
R31	2*	24
R26-28	**16**	**43.4±13.3**
R29+30	8	39±14
R29+30+31	**10**	**35.7±13.8**
R26=31	26	40±14

* One result was dropped as a clear outlier (R26: 56, 64, 54, 139), (R31: 202, 23, 25).

** Controls were born in 1963 or later.

TABLE 3

Controls (all 28 samples)

ID#	1st drop	2nd drop	Blank	Net tracks
R26				
FUB1	30	29	12	35
FUB2	54	25	9	61
FUB3	21	24	9	35
FUB4	26	35	6	49
JUB1	34	28	3	56
JUB2	46	26	4	64
JUB3	31	31	4	54
JUB4*	125	40	3	159
WUB1	30	33	8	47
WUB3	28	24	9	34
WUB4	23	39	6	50
R28				
KUB11	30	16	10	26
KUB12	30	17	10	27
KUB13	50	41	16	59
FUB13	33	28	10	41
FUB14	30	19	13	23
FUB15	21	24	6	33
R29				
FUB16	44	21	4	57
BUB1	53	20	9	55
STUB1	22	20	8	26
KUB14	36	34	15	40
R30				
FUB17	43	20	10	43
KUB15	31	35	11	44
STUB3	31	25	16	24
BUB2	19	17	8	20
R31				
FUB18*	114	110	11	205
KUB16	24	23	12	23
BUB3	32	21	14	25

* result was dropped as a clear outlier

Results for Reagent Blanks and Comparison with the Results for Control Urines:

The results of 18 reagent blank determinations are listed in Table 4. The reagent blanks sometimes consisted of processing reagents through both columns and sometimes through column 2 only, since we found in a subset of samples that there was no significant difference between the results for processing through 2 columns and the second column only. The mean of 18 reagent blank determinations was 31.8 tracks with a standard error of the man of 2.4. Normality was not rejected (p=0.33) and comparison by a t-test with the mean from the 26 control samples (40±14) showed a statistically significant difference (p=0.029) of 9 tracks. We cannot yet say whether these 9 tracks were due to uranium or plutonium, but if they were due to plutonium in the control urine samples, they would be equivalent to 6 aCi of ^{239}Pu or about 30 aCi/liter. Ideally, if there is no plutonium in control urine samples, then the number of tracks in reagent blanks should be equal to the number of tracks in control urine.

Demonstration of Feasibility of Measuring 10 aCi/l in Human Urine:

During the year, we gradually increased the volume of sample analyzed, first experimenting with 0.8 liter volumes of urine and, more recently, with 8 liter samples. The reason for choosing the large voulumes was that our calculations indicated that there should be a background of ^{239}Pu on the order of 25 aCi/l in normal human urine. The basis for this estimate is shown in Table 5. Table 6 shows the method by which the calculation of activity and its standard deviation was made for urine samples.

These 8 liter samples were taken from two sets of people whose ages are shown in Table 7. In order to obtain 8 liters in a timely manner, it was necessary to composite urine from two young person and two middle aged person. In any future studies, there will be no compositing between people so that individual samples will reflect an individual person. Rundo, who measured urine from people who had been injected with ^{239}Pu approximately 10,000 days earlier showed that a mean of about 2 x $10^{-5}$of the body burden is excreted daily in urine. With a mean body burden of 3 pci of ^{239,240}Pu in the general population, excretion in urine should be about 60 aCi/day or about 43 aCi/l if 1.4 liters of urine is excreted daily. Of this 43 aCi/l, only 60% is ^{239}Pu, therefore, the excretion of ^{239}Pu in human urine should be equal to 26 aCi/l.

Figures 2, 3 and 4 show the elution pattern for the young subjects, middle aged subjects and synthetic urine. The elution pattern characteristic of Pu was found in all three samples, a surprising result, although in the synthetic urine, the activity was much lower than in the middle aged subjects or in the young subjects. The preliminary results indicate that activity estimated for yourtg, middled-aged and synthetic urine was 33, 50 and 11 aCi/l liter of urine respectively (Table 7). These values should be considered approximate since the efficiency of yield may have been slightly higher for these samples than in previous samples because a larger volume was eluted from the second column and, therefore, a larger fraction of plutonium from the column.

In order to quantify the analysis, we subtracted a reagent blank background of 32 tracks to obtain net tracks in the samples. It seems inappropriate that the synthetic urine should be used for background subtraction, since the reagents that make up the synthetic urine differ from the reagents that are used in the processing of normal human urine. For that reason, we have subtracted the reagent blank background lieu of the sunthetic urine background. Also, this only represents one sample each of 8 liters. An 8 liter sample is very time consuming to process at the moment and work is underway to decrease the amount of time required to process such samples. However, attempts are being made to find the optimum volume of urine samples which may be easy to handle yet yield a sufficient number of tracks due to normal levels of ^{239}Pu present in urine.

Acknowledgement: Thanks are due to the U. S. Department of Energy (DOE office at Las Vegas), and the Office of Epidemiology (DOE Headquarters) for funding the project through contract number DE-FC08-89NV10865.

TABLE 4

Reagent Blanks (all 18 samples)

	ID#	1st drop	2nd drop	Blank	Net tracks
R24					
	RB1	19	26	8	29
	RB2	20	40	7	46
	RB3	19	32	7	37
	RB4	15	17	10	12
R26					
	RB1	35	28	16	31
	RB2	42	36	16	46
	RB3	29	28	8	41
	RB6	24	31	9	37
R28					
	RB1	21	33	10	34
	RB2	16	44	12	26
	RB3	31	36	11	45
	RB4	35	38	24	26
R29					
	RB1	17	19	6	24
	RB2	33	29	10	42
	RB3	16	16	8	16
	RB4	20	17	7	23
R30					
	RB1	20	25	5	35
	RB2	25	19	11	22

Mean ± SE = 31.8 ± 2.4

TABLE 5

Rundo measured the daily urinary excretion of two subjects at 10,000 days post injection of 0.3 μCi ^{239}Pu citrate and found:

Female (77)	2.52×10^{-5}
Male (73)	1.41×10^{-5}
mean ~	2×10^{-5}

We know the body burden of our adult subjects should be about:

3×10^{6} aCi. ($^{239+240}$Pu)

Thus, daily excretion in urine should be:

$3 \times 10^{6} \times 2 \times 10^{-5} = 60$ aCi/day

If daily urinary output is 1.4 liter,
then excretion/l = 42.8 aCi/l $^{239+240}$Pu.

For global fallout ^{240}Pu/^{239}Pu = 0.18,
and the ^{239}Pu activity = 0.60 $^{239+240}$Pu.

So: Predicted ^{239}Pu excretion = 0.60 x 42.8 = 25.7 aCi/l

TABLE 6

CALCULATION OF ACTIVITY AND STOCHASTIC ERROR IN NET ACTIVITY FOR EACH BNL SAMPLE

The activity associated with the activity in a sample is given by: $A = \frac{(N-C)}{eYV}$

where

A	stands for net activity per liter (aCi/l)
N	stands for net tracks in sample (i.e. $N = n_1 + n_2 - 2n_3$)
C	stands for net tracks in controls
n_i	stands for gross tracks in drop i, where i = 3 refers to background
V	stands for volume of samples analyzed (0.2 liter)
eY	stands for tracks per aCi in sample (product of yield and effiency)

The error associated with the activity in each sample is found as follows:

$$\sigma^2 = \sigma^2{}_N + \sigma^2{}_C$$

The standard deviation of N is taken as Poisson, namely $\sigma^2{}_N = n_1 + n_2 + 2n_3$; σc was calculated from n measurements of C according to the usual formula $\sigma^2{}_C = \Sigma \frac{(C-Ci)^2}{n-1}$

Where there were replicate measurements N_1 and N_2, $N = (N_1 + N_2)/2$
and

$$\sigma^2{}_N = (\sigma^2{}_{N1} + \sigma^2{}_{N2})/2$$

and

$\sigma A = \frac{\sigma}{(1.62)(0.2)}$ is the estimate of standard deviation for the estimate of activity/liter.

LEXAN SLIDE

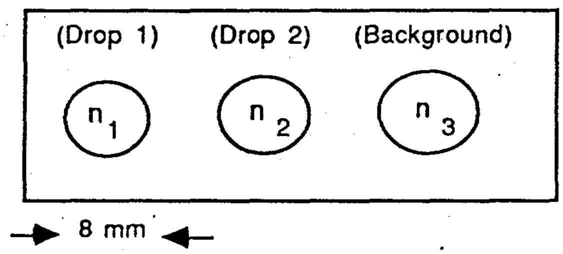


TABLE 7

MEASUREMENT OF 239Pu IN URINE OF 2 NON-OCCUPATIONALLY EXPOSED PERSONS AND SYNTHETIC URINE

Sample Size: 8 liters

(Y) Young person composite: 2 aged (21 yrs. and 26 yrs.)
(M) Middle aged: 2 aged (49 yrs. and 49 yrs.)
(S) Synthetic Urine

	TRACKS		
	Y	M	S
1st drop	232	373	101
2nd drop	159	283	54
3rd drop	72	47	23
4th drop	14	7	21
Blank #1	5	9	5
Blank #2	6	7	10

NET TRACKS: 455 678 169

Use the reagent blank background of 32 tracks

e = 1.6 tracks/aCi

$^{239}Pu_y$ = (455 - 32)/(1.6 x 8) = 33.0 aCi/l
$^{239}Pu_m$ = (678 - 32)/(1.6 x 8)= 50 aCi/l
$^{239}Pu_s$ = (169 - 32)/(1.6 x 8)= 11 aCi/l

FIGURE 1

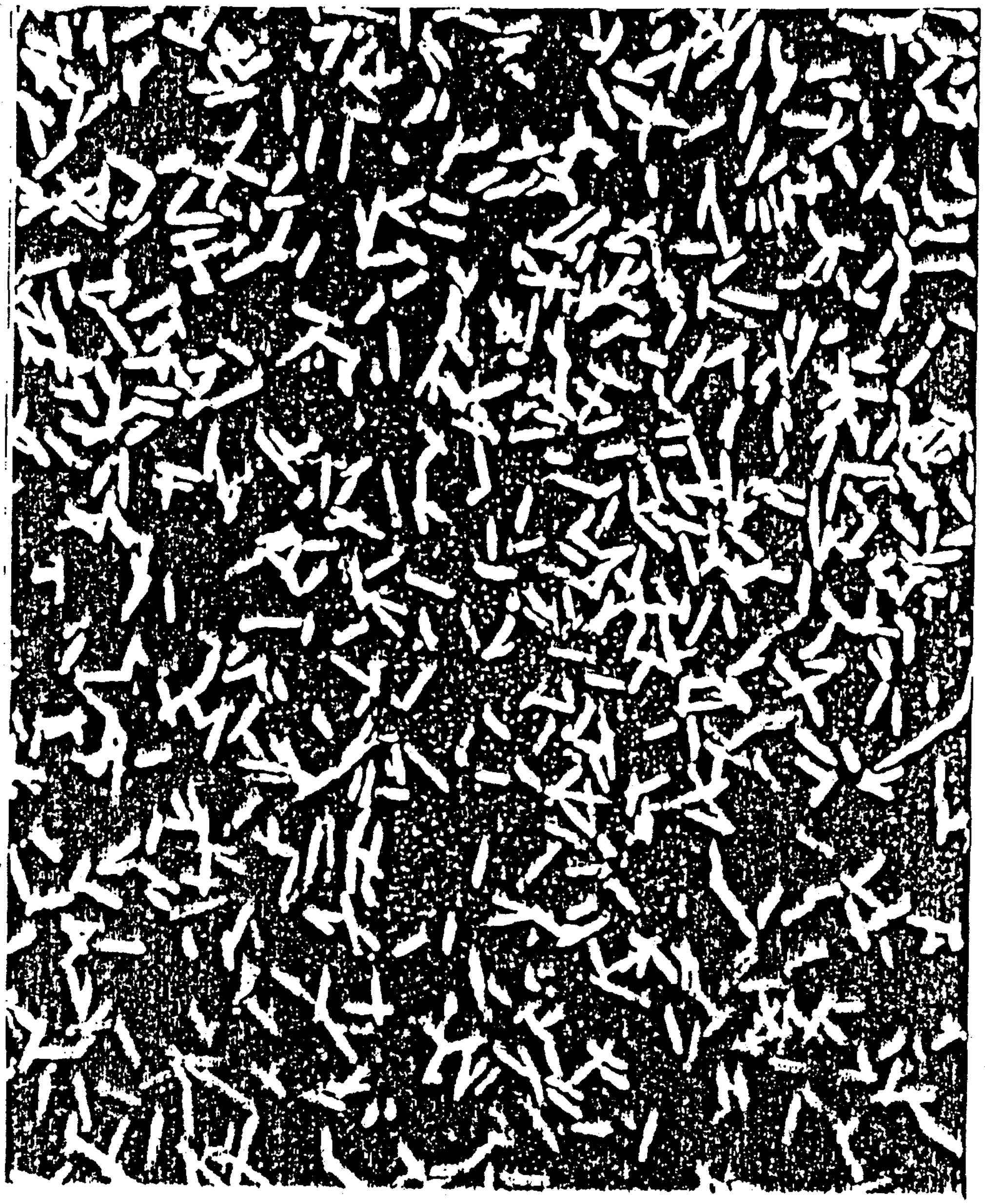


FIGURE 2

Young subject, 8L sample

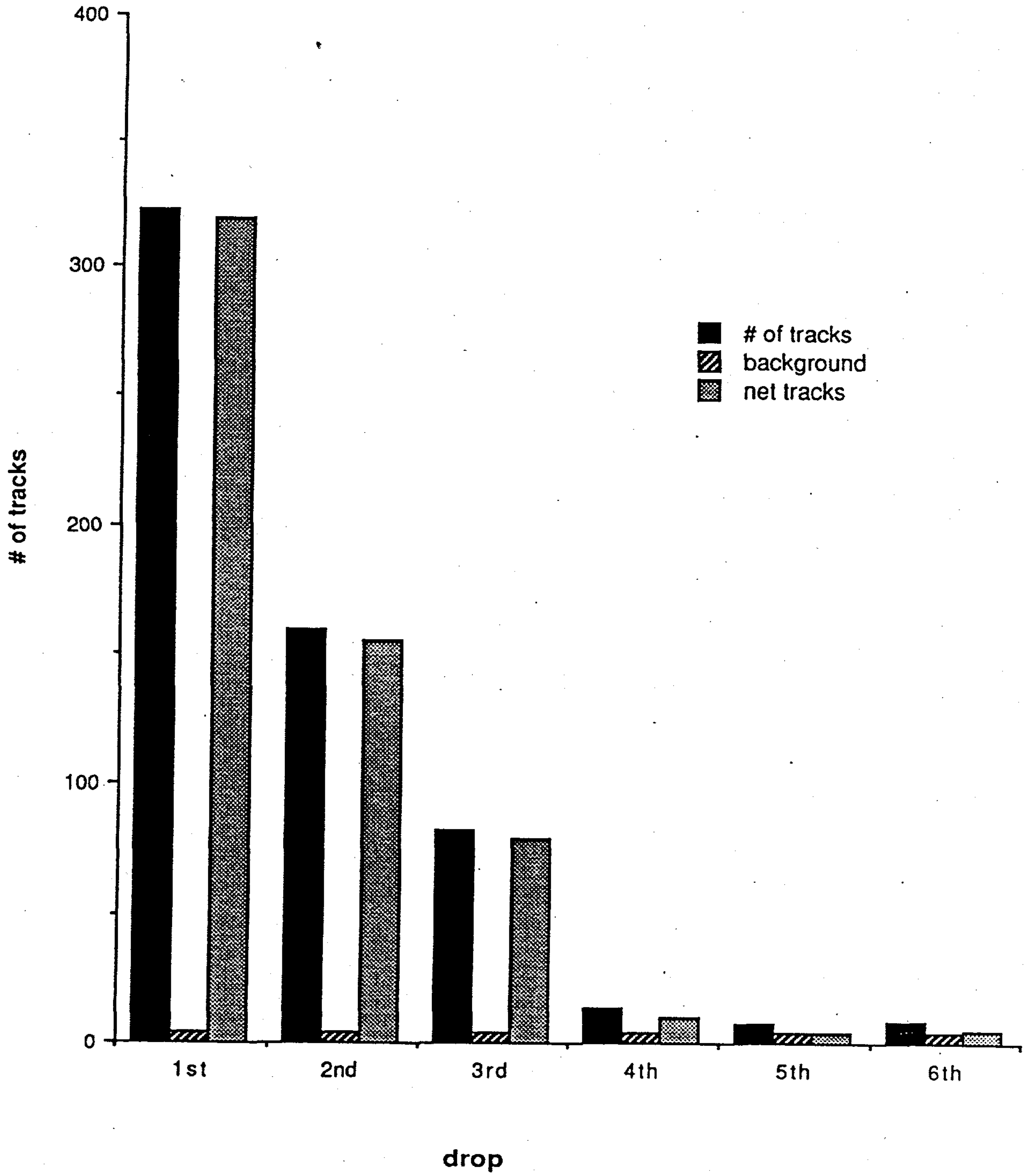


FIGURE 3

Mid-aged Urine, 8L sample

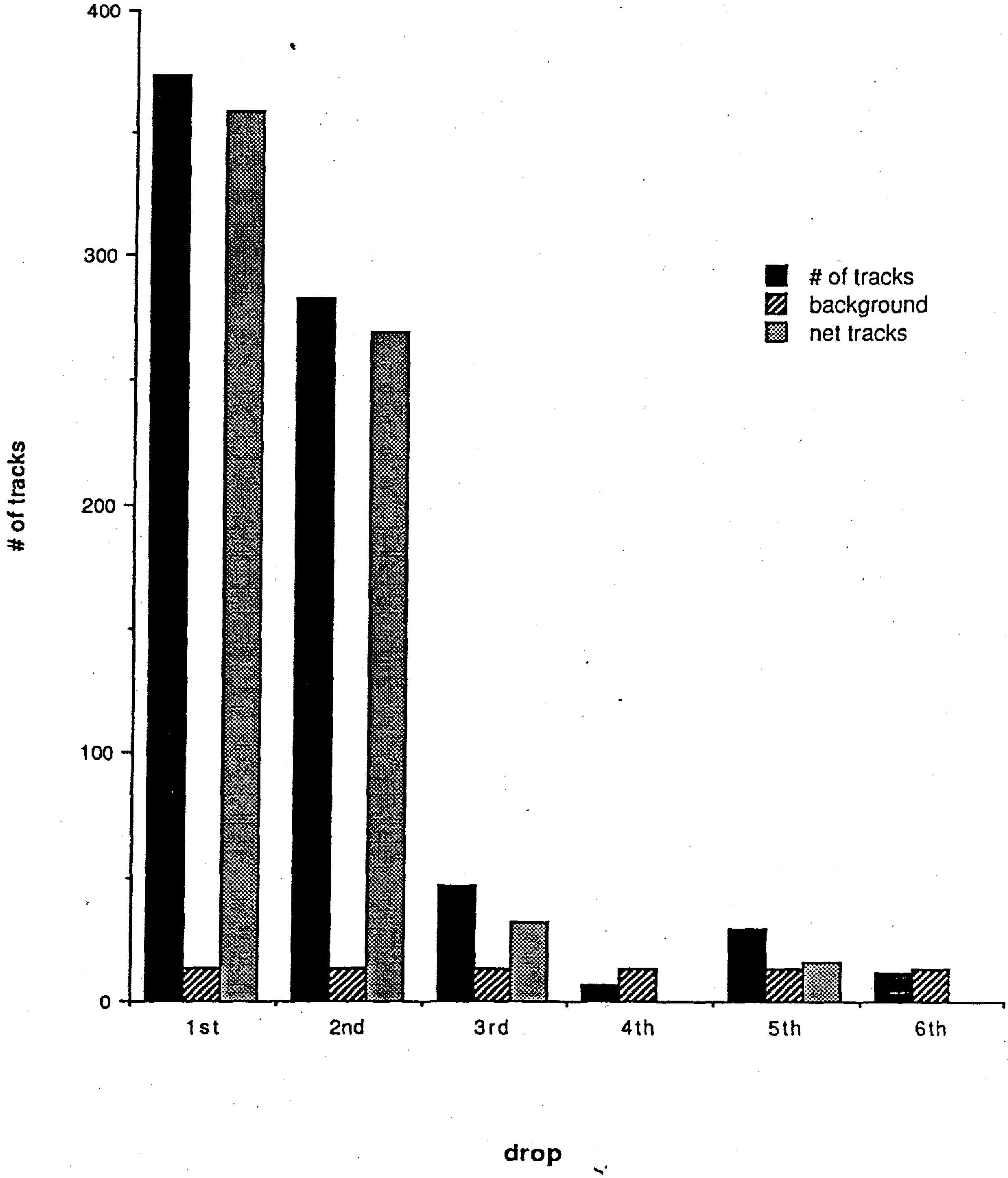
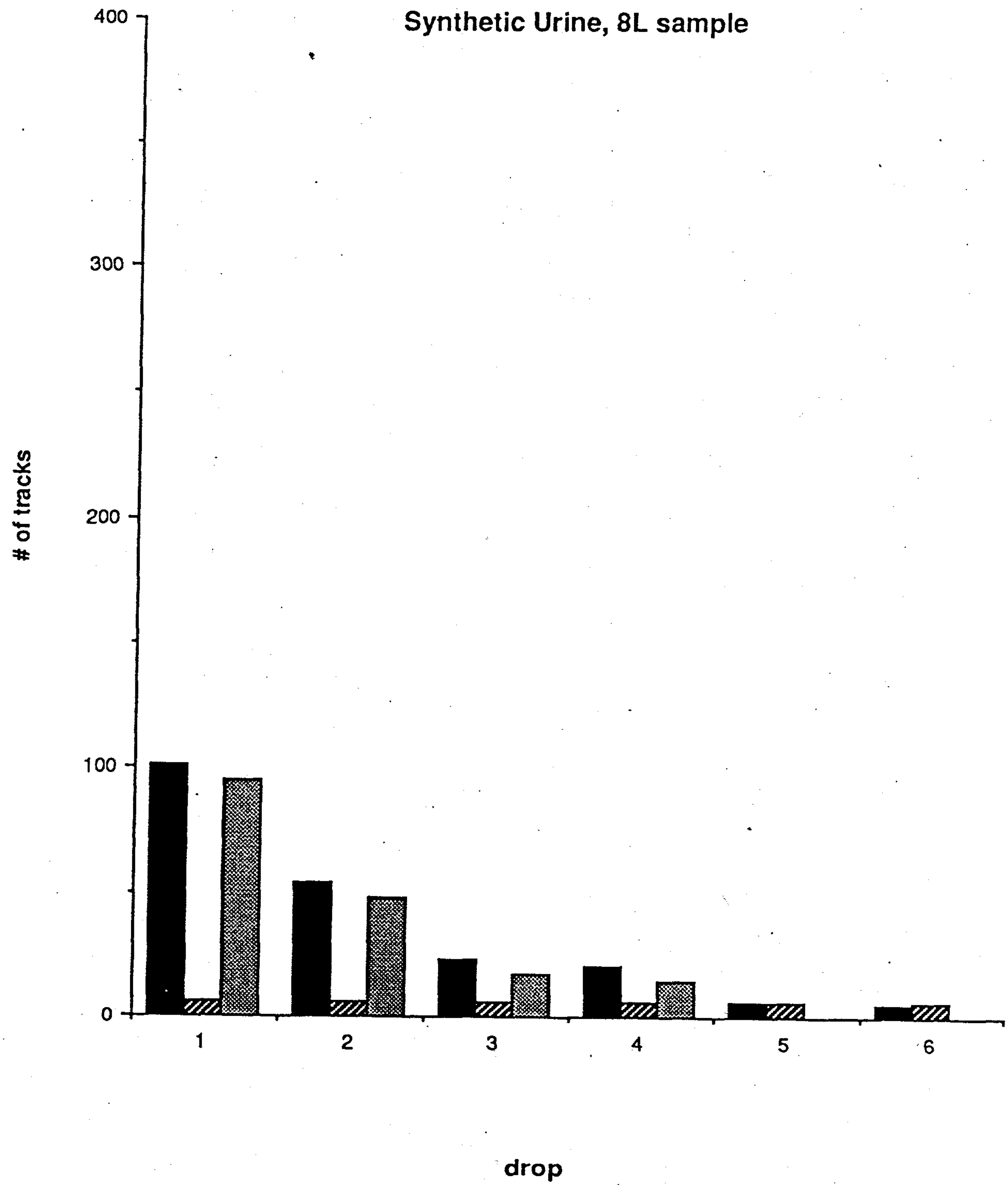


FIGURE 4


Synthetic Urine, 8L sample
# of tracks
400
300
200
100
0
1
2
3
4
5
6
drop