which gave a major single fluorescent spot on TLC under UV light together with traces of five nonfluorescent components. Subsequent isolation of this single fluorescent spot by preparative TLC led to a crystalline residue, which was recrystallized from benzene to yield colorless needles (8 mg), mp 170–173°. The identity of this compound with an authentic sample of helenalin (I) was established by TLC, IR, and NMR spectroscopic comparisons and by mixed melting-point determination.

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Polyamine Metabolism II: *N*-(Monoaminoalkyl)- and *N*-(Polyaminoalkyl)acetamides in Human Urine

MAHMOUD M. ABDEL-MONEM ˣ and KOSEI OHNO

Abstract □ TLC and high-pressure liquid chromatographic examination of the dansyl derivatives obtained from human urine indicated the presence of *N*-[3-[(4-aminobutyl)amino]propyl]acetamide (N^1-acetylspermidine), *N*-[4-[(3-aminopropyl)amino]butyl]acetamide (N^8-acetylspermidine), *N*-(4-aminobutyl)acetamide (*N*-acetylputrescine), and *N*-(5-aminopentyl)acetamide (*N*-acetylcadaverine). The ratio of N^1- to N^8-acetylspermidine ranged from 10.3 in the urine of a patient with hepatoma to 1.1 in the urine of a normal subject. The three cancer patients had a considerably higher ratio of N^1- to N^8-acetylspermidine than did the three normal subjects. These findings indicate that the ratio of N^1- to N^8-acetylspermidine in the 24-hr urine may serve as a biochemical marker for cancer.

Keyphrases □ Polyamines—*N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, dansyl derivatives, TLC and high-pressure liquid chromatographic analysis, human urine □ *N*-(Aminoalkyl)acetamides—dansyl derivatives, TLC and high-pressure liquid chromatographic analysis, human urine □ Dansyl derivatives—*N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, TLC and high-pressure liquid chromatographic analysis, human urine □ TLC—analysis, dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, human urine □ High-pressure liquid chromatography—analysis, dansyl derivatives of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides, human urine

The diamine 1,4-butanediamine (putrescine) (I) and the polyamines *N*-(3-aminopropyl)-1,4-butanediamine (spermidine) (II) and *N,N'*-bis(3-aminopropyl)-1,4-butanediamine (spermine) (III) are excreted in human urine mainly in the form of conjugates which produce the free amines after hydrolysis (1). *N*-(4-Aminobutyl)acetamide (*N*-acetylputrescine) (IV) was identified in the urine of normal subjects (2), and *N*-(5-aminopentyl)acetamide (*N*-acetylcadaverine) (V) was identified in the urine of

$RHN(CH_2)_nNH_2$ $\qquad$ $RHN(CH_2)_3NH(CH_2)_4NH_2$

I: R = H, $n = 4$ $\qquad$ II: R = H

IV: R = CH_3CO, $n = 4$ $\qquad$ VI: R = CH_3CO

V: R = CH_3CO, $n = 5$

$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

III

$CH_3COHN(CH_2)_4NH(CH_2)_3NH_2$

VII

schizophrenic patients (3). *N*-[3-[(4-Aminobutyl)amino]propyl]acetamide (N^1-acetylspermidine) (VI), but not *N*-[4-[(3-aminopropyl)amino]butyl]acetamide (N^8-acetylspermidine) (VII), also was detected in urine of healthy children (4) and a patient with acute myelocytic leukemia (5). Recently, both VI and VII were identified in urine of normal subjects and cancer patients (6, 7).

The present paper reports the determination of the ratio of VI to VII in the urine of normal subjects and cancer patients who had not received therapy.

EXPERIMENTAL

Extraction of Polyamines and Conjugated Polyamines from Urine—Twenty-four hour urine samples were collected from three adults and one child as normal subjects and from three patients with diagnosed cancer (hepatoma, melanoma, and thyroid cancer) who had not received therapy. The urine was collected over toluene, kept refrigerated during collection, and stored at −20° until analysis.

An aliquot of urine (100 ml) was adjusted to pH 10–11 with 2 *N* NaOH (5 ml) and extracted with 2-methyl-1-butanol (4 × 50 ml). Nitrogen was bubbled through the organic solvent extract to remove ammonia. Concentrated hydrochloric acid (5 ml) was added to the extract, and the mixture was evaporated to dryness *in vacuo* at 45°.

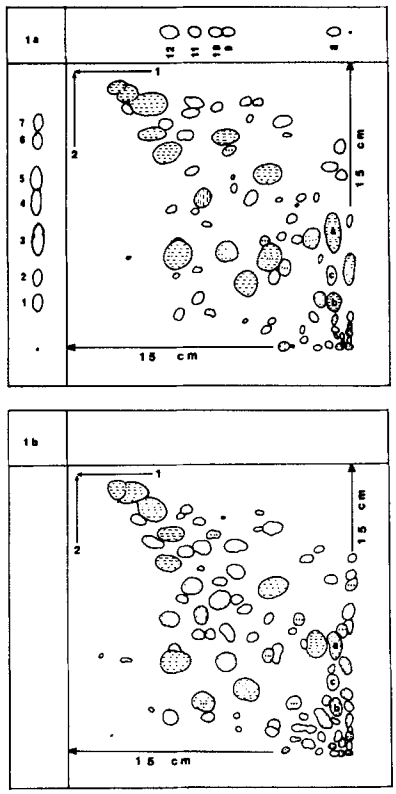

Figure 1—*Two-dimensional TLC of the dansyl derivatives obtained from the urine of a patient with hepatoma* (a) *and a normal subject* (b). *Compounds tested were the dansyl derivatives of: IV (1); V (2); VI, VII, N^4-acetylspermidine, and ammonia (3); I, 1,3-propanediamine, and N^4-acetylspermine (4); N^1-acetylspermine (5); II (6); III (7); IV, V, acetylspermidines, and acetylspermines (8); III (9); II (10); 1,3-propanediamine and I (11); ammonia (12); VI and VII (spot a); IV (spot b); and V (spot c).*

Formation of 5-(Dimethylamino)-1-naphthalenesulfonyl (Dansyl) Derivatives of Urine Extracts—The residue obtained after evaporation of the solvent was dissolved in 2% HCl (5 ml), and then the flask was rinsed with water (5 ml) and acetone (15 ml). The solution was adjusted to pH 8.5–9 with anhydrous sodium carbonate (about 2.0 g) and then treated with dansyl chloride (26.9 mg, 0.1 mmole) in acetone (10 ml).

The mixture was stirred at room temperature for 12 hr in a covered flask and concentrated to remove the acetone. Water (30 ml) was added to dissolve the precipitated solids, and the reaction mixture was extracted with chloroform (4 × 25 ml). The combined chloroform extract was washed with water (10 ml) and dried over anhydrous sodium sulfate. The extract was evaporated to dryness *in vacuo*.

Isolation and Purification of Dansyl Derivatives of *N*-(Monoaminoalkyl)- and *N*-(Polyaminoalkyl)acetamides—The residue obtained from the dansylation reaction was dissolved in 1.0 ml of chloroform, and 10 μl of this solution was applied to a TLC silica gel GF plate[1] (250 μm, 20 × 20 cm). A mixture of the authentic samples of the dansyl derivatives of the *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides also was spotted on some plates for comparison. Each plate was developed using cyclohexane–ether (1:9) for an elution distance of 14 cm and then in a second dimension using chloroform–triethylamine (10:1). The plates were examined under a long wavelength UV lamp.

The desired spots were scraped separately from the plates and immediately extracted with a mixture of equal volumes of triethylamine and 2-propanol (10 ml). The extract was evaporated to dryness in a nitrogen stream at 40°, and the residue was stored in a refrigerator until analysis by high-pressure liquid chromatography (HPLC). Immediately before HPLC analysis, the residue was dissolved in 50 or 25 μl of chloroform, and a 5- or 10-μl aliquot was injected.

Determination of Dansyl Derivatives Using HPLC[2]—The separation of the dansyl derivatives was achieved, using a modification of a published method (8), on a silica column[3] (120 cm × 2.2 mm i.d.) with chloroform (distilled over anhydrous calcium chloride)–2-propanol (100:6).

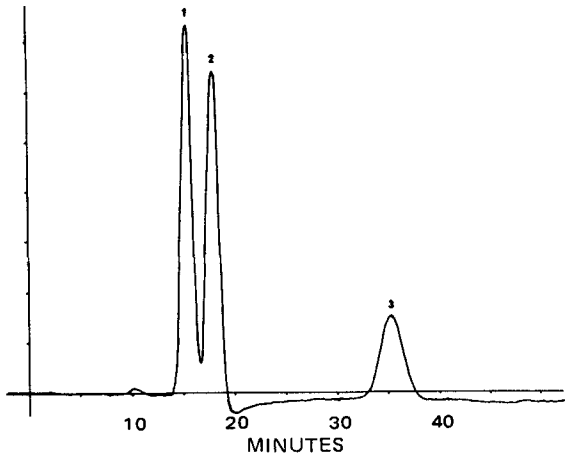

Figure 2—*HPLC separation of a standard solution containing 100 pmoles of each dansyl derivative of: VI (1), VII (2), and IV (3).*

RESULTS

Typical chromatograms of the TLC separation of the dansyl derivatives from the urine of a patient with hepatoma and a normal subject are shown in Figs. 1*a* and 1*b*, respectively. Cochromatography of a mixture of authentic samples of IV–VII permitted the identification of the position of each compound on the chromatogram of the derivatives from urine.

A typical HPLC separation of a mixture of IV, VI, and VII (100 pmoles of each) is shown in Fig. 2. Different amounts of each dansyl derivative of *N*-(monoaminoalkyl)- and *N*-(polyaminoalkyl)acetamides were injected, and a linear correlation was observed between the peak height and the amount injected for each compound.

Figures 3 and 4 represent the chromatograms obtained by HPLC examination of extracts from the spots corresponding to a mixture of VI and VII (spot a) and IV (spot b), respectively, on the thin-layer chromatogram of the dansyl derivatives from urine of a hepatoma patient (*a*) and a normal subject (*b*).

The concentration of each dansyl derivative was calculated by comparison of its peak height with that obtained from known concentrations of an authentic sample. Table I presents the quantitative results obtained for three normal subjects, one child, and three cancer patients. The values were not corrected for losses during the analytical procedure and may be underestimates of the actual values.

DISCUSSION

TLC and HPLC examination of the dansyl derivatives of the amines

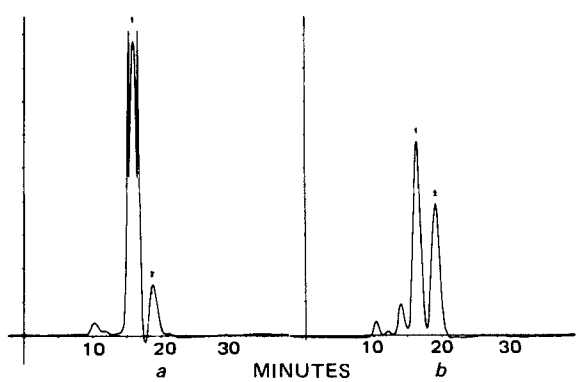

Figure 3—*HPLC separation of the dansyl derivatives of the monoacetylspermidines obtained from the urine of a patient with hepatoma* (a) *and a normal subject* (b). *Key: 1, VI; and 2, VII. Peak intensity for VI in a was reduced to one-half its size for comparison. The amount injected in a was one-half the amount injected in* b.

[1] Analtech, Newark, Del.

[2] Model 6000 solvent delivery system, Waters Associates, Milford, Mass., and LDC model 1209 fluoroMonitor, Laboratory Data Control, Riviera Beach, Fla.

[3] Corasil II, Waters Associates, Milford, Mass.

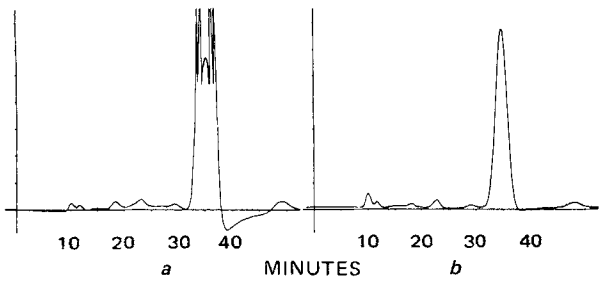

Figure 4—*HPLC separation of the dansyl derivatives of IV obtained from the urine of a patient with hepatoma* (a) *and a normal subject* (b). *The peak intensity for IV in* a *was reduced to one-fourth its actual size for comparison.*

in human urine indicated the presence of IV–VII in appreciable amounts in all samples examined. *N*-(4-Aminobutyl)-*N*-(3-aminopropyl)acetamide (N^4-acetylspermidine) and the two isomeric N^1- and N^4-monoacetyl derivatives of III were not present in detectable amounts. The identity of IV, VI, and VII obtained from human urine was confirmed by mass spectral analysis (7). Compound V was present in low concentrations and was not quantified.

In the seven samples examined, the ratio of VI to VII was greater than 1. The highest ratio was observed in the urine of a patient with hepatoma (10.3); the lowest ratio was observed in the urine of a normal subject (1.1). The considerably higher levels of VI in the urine of the hepatoma patient is noteworthy. The three cancer patients had a considerably higher ratio of VI to VII than did the three normal subjects. These findings indicate that normal subjects and cancer patients not only differ in their levels of urinary polyamines (1) but also in the nature of the polyamine conjugates. Moreover, the ratio of VI to VII in the 24-hr urine may serve as a biochemical marker for cancer and is being further studied in this laboratory.

A ratio of concentration of VI to VII greater than 1 indicates a higher rate of formation and/or a lower rate of degradation of VI relative to VII. Unfortunately, little is known about the enzymes responsible for the formation of these acetylated aliphatic amines and their fate in mammalian tissues. Recently, Seiler and Al-Therib (9) described the *in vitro* acetylation of I to IV catalyzed by acetyl coenzyme A putrescine *N*-acetyltransferase from rat tissues. This enzyme was specific for the acetylation of I; the highest activity was observed in the brain, especially in the nuclei.

Spermidine and spermine did not inhibit the enzymatic acetylation of putrescine, indicating that these compounds did not act as substrates for this enzyme and that the enzymes responsible for acetylation of the diamines and polyamines possess a high degree of substrate specificity. Therefore, mammalian tissues may conceivably contain separate enzymes that acetylate spermidine to form either VI or VII and cancer tissue may have higher activity of the N^1-spermidine *N*-acetyltransferase. On the other hand, Seiler and Al-Therib (10) showed that IV was oxidatively deaminated both *in vitro* and *in vivo* by monoamine oxidase. It is possible, therefore, that the acetylspermidines also are oxidatively deaminated by amine oxidases and that the higher ratio of VI to VII in cancer tissues reflects a higher rate of degradation of VII in these tissues.

Table I—Concentrations of *N*-(Monoaminoalkyl)- and *N*-(Polyaminoalkyl)acetamides in Urine of Normal Subjects and Cancer Patients

Subject	Micromoles per 24 hr[a]			
	VI	VII	VI/VII	IV
Normal adult	0.277	0.237	1.17	1.503
Normal adult	0.135	0.118	1.14	1.181
Normal adult	0.735	0.562	1.31	3.613
Child[b]	0.115	0.070	1.64	0.569
Thyroid cancer patient	0.403	0.229	1.76	2.587
Melanoma patient	1.075	0.610	1.76	5.800
Hepatoma patient	4.413	0.428	10.31	12.810

[a] Values are not corrected for losses during the analytical procedure. [b] Micromoles per 100 ml for the urine sample voided in the morning (110 ml).

The concentration of IV was 2.6–4.7 times greater than the total amount of the isomeric acetylspermidines. There was an apparent linear correlation (r = 0.970) between the levels of IV and those of the acetylspermidine in urine.

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