Catabolism and Excretion of Fibrinopeptide-A

By Norma Alkjaersig and Anthony P. Fletcher

Mean urinary excretion of fibrinopeptide-A (FPA) in 51 normal individuals ranging in age from 7 to 92 yr was 1.67 ± .1 (SE) μg/24 hr with no significant differences related to age or sex, though children tended to excrete less FPA. Calculation suggests that FPA urinary excretion represents 0.2%–0.5% of total FPA released from fibrinogen/24 hr. Urinary FPA eluted from gel permeation columns in the same position as 125I-FPA. 125I-FPA (human) was cleared from dog plasma with a half-life of 3.9 mm. and 0.5% of the total dose could be accounted for in the urine over a 3-hr period. FPA assay in paired plasma and 24-hr urine samples from 15 normal individuals, 16 patients with malignancy, 3 patients with other disease, and 5 patients with severe burns (several samples from each) demonstrated significant correlation between plasma FPA concentration and 24-hr urinary FPA excretion (p < 0.001).

FIBRINOPEPTIDE-A (FPA) is a small molecule, and possibly its rapid disappearance from plasma over a 24-hr period. FPA is a small molecule, and possibly its rapid disappearance from plasma may be partially due to excretion in the urine. Attempts to detect urinary excretion of FPA in dogs undergoing experimental intravascular coagulation and endotoxin-treated rabbits have been largely unsuccessful. These investigators used chemical assay methods, which are less sensitive than current radioimmunoassay methods. However, in animal studies where radiolabeled FPA was injected intravenously, urinary FPA excretion was observed to be less than 1% of the injected dose.

The development by many investigators, particularly by Nossel et al., of radioimmunoassay procedures for FPA has increased assay sensitivity to the picomole level. Consequently, considerable interest in fibrinopeptide-A assay as a tool to elucidate thromboembolic vascular disease pathophysiology has been manifest. However, interpretation of such studies has been handicapped, because the short plasma half-life of FPA has raised doubt as to the value of a single FPA determination as an overall measure of thrombin action on fibrinogen.

In this article we describe studies on FPA urinary excretion in normal subjects and in patients.

MATERIALS AND METHODS

Urine

Urine was collected over a 24-hr period from normal individuals and from patients, some of whom were catheterized. No preservatives were added. Volume and pH were recorded, and pH was adjusted to 8.5 before assay; urine samples were also tested for the presence of blood with the use of Multistix (Ames Division, Miles Laboratories, Elkhart, Ind.). FPA assays were performed at the end of the collection period, except in the burn patients, where urine aliquots were frozen (–70°C) prior to assay.

Venous Blood

After clean venipuncture, venous blood was collected into vacutainer tubes containing the proprietary anticoagulant (EDTA, aprotinin, and a thrombin inhibitor) supplied with the Mallinckrodt FPA assay kit. FPA in plasma containing this anticoagulant stored at –70°C was stable; however, repeated freezing and thawing tended to increase assay values. Plasma kept at room temperature for 16 hr showed a mean FPA increase of 32%; the increase could be abolished by additional aprotinin (0.1 trypsin inhibitor U/ml), Sigma, in this case FPA was reduced by 8%. In a few instances, blood was collected by two-syringe technique and using the heparin-protamine anticoagulant mixture suggested by Nossel et al. Patient plasma samples were drawn in the morning and assayed the same day, except for the burn patients where plasma samples were frozen immediately after centrifugation. Patients and normal individuals all gave informed consent for venipuncture, and the study was approved by the institutional Human Studies Committee.

Fibrinopeptide-A Assay

Fibrinopeptide A assay was performed with an assay kit supplied by Mallinckrodt, St. Louis, Mo. The kit supplies 125I-tyrosylated...
FPA (specific activity 2000 Ci/mmole), a dilution series of unlabelled FPA, and bentonite for plasma adsorption. Bound radioactivity is removed by the double antibody technique; both antibodies are also supplied in the kit. The kit is convenient to use, and assay results are available within 2–3 hr. Comparative assays were performed with the Mallinckrodt kit and with the IMCO (Stockholm, Sweden) kit, the assays being performed according to the respective manufacturer's instructions, except that bentonite adsorption was used, as suggested by Wilner,6 instead of alcohol precipitation-dialysis suggested by IMCO. Almost identical results were obtained by the two methods, the correlation coefficient was 0.99 between the two sets of assays. A fibrinogen solution, 3.2 mg/ml Tris buffer, was distributed into aliquots and frozen. Over a period of 2 mo, 12 aliquots, suitably diluted, were treated with thrombin (Parke Davis) adsorbed with bentonite and assayed for FPA. Thrombin concentration was 10 U/ml for 20 min. The thrombin, assayed in a concentration of 100 U/ml, had no FPA activity. The results were 31.85 ± 1.88 (SD) μg FPA/ml. Three quality controls (0.5, 2.5, and 9.5 ng/ml) assayed over the same time period had a coefficient of variation of 12% or less. The FPA antiserum was tested for cross-reactivity with fibrinogen as described by Canfield et al.4 and was approximately 4%.

Chromatography

Chromatography was performed on Sephadex G-25 (0.9 x 40 cm) columns, equilibrated and eluted with 0.06 M Tris, 0.15 M NaCl buffer of pH 8.5 containing 1 mg/ml ovalbumin (Sigma). When necessary, urine samples were concentrated by pervaporation before being applied to the column. Most urine samples were assayed with and without bentonite adsorption, and several plasma samples and most urine samples were assayed with and without thrombin treatment, final concentration (10 U/ml for 20 min).

Dialysis

Two milliliters each of paired urine and plasma samples were dialyzed against 2 ml Tris-albumin buffer, pH 8.5, with a 1:10 dilution of the anticoagulant supplied with the Mallinckrodt kit with constant agitation for 90 hr in the cold.

Clearance of 125I-FPA (Human) From Dog

Approximately 10 ng 125I-FPA was passed through a Sephadex G-25 column; the peak fractions were combined (total count 106 cpm) and injected intravenously into an anesthetized and catheterized 17 kg dog. Blood samples were drawn every 2 min for 20 min and a final sample at 3.5 hr; after initial bladder washout, urine was collected every 30 min for 3.5 hr. FPA is stable in rabbit urine.4

Stability Studies

Urine, containing 19 ng FPA/ml, was divided into 5 aliquots, adjusted, respectively, to pH 5, 6, 7, 8, and 9. Aliquots from each of these were placed at –70°C, room temperature (range 20°–27°C), and at 37°C. Each aliquot was assayed on days 0, 1, 2, and 5.

Blood in Urine

Whole blood was added to urine in the following concentrations, 10 and 1 μl and 100, 10, 1, and 0.1 μl/ml urine. Each sample was tested by Multistix and assayed for FPA directly and after thrombin treatment.

RESULTS

FPA assays in urine samples frozen at –70°C showed no change after 1-wk storage (data not shown). Figure 1 shows FPA stability in urine samples incubated at room temperature and 37°C and adjusted to pH between 5 through 9. The figure shows that FPA stability in urine at room temperature is greatest at pH 5.0 and less between pH 6 and 8, the largest loss being at pH 7 (12% over 24 hr). Substantially less stability was seen at 37°C. Less complete FPA stability studies with 16 other urines, over a wide range of FPA concentration, gave essentially similar results to those shown in Fig. 1. Half of the urines tested in this study were of pH less than 6, and 40% were of pH 6–8. Thus, assay error due to FPA instability would be expected to be minor, even if the urine collection bottle was kept at room temperature during the collection period as it was in most instances. Diurnal variation of FPA excretion was not observed in two individuals in whom samples were collected at 4-hr intervals over 24 hr.

Urinary tract bleeding would lead to artifically high FPA excretion values as was observed in a patient with renal calculi and some of the catheterized patients. Consequently, all urine samples were tested with Multistix, and samples giving a positive reaction for blood were excluded from analysis except when stated. Table I shows that the sensitivity of Multistix is adequate to exclude significant contamination with blood. The manufacturer claims that 0.015–0.045 mg/dl of free hemoglobin (or 5–15 intact erythrocytes/μl) in urine can be detected, this is equivalent to ~3 μl blood/ml urine or, assuming normal fibrinogen level in the blood, 5 ng/ml fibrinogen with an FPA.

Table 1. Addition of Whole Blood to Urine

<table>
<thead>
<tr>
<th>Blood/ml Urine</th>
<th>Multistix Reading</th>
<th>No Thrombin</th>
<th>With Thrombin</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μl</td>
<td>Large</td>
<td>5</td>
<td>251</td>
</tr>
<tr>
<td>1 μl</td>
<td>Moderate</td>
<td>2.5</td>
<td>29.8</td>
</tr>
<tr>
<td>0.1 μl</td>
<td>Small</td>
<td>1.8</td>
<td>4.7</td>
</tr>
<tr>
<td>10 nl</td>
<td>Trace</td>
<td>1.44</td>
<td>2.3</td>
</tr>
<tr>
<td>1 nl</td>
<td>Trace</td>
<td>1.42</td>
<td>1.5</td>
</tr>
<tr>
<td>0.1 nl</td>
<td></td>
<td>1.37</td>
<td>1.47</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>1.50</td>
<td>1.46</td>
</tr>
</tbody>
</table>

Fig. 1. Stability of FPA in urine at pH ranging from 5 to 9 and at room temperature (Φ—Φ) or 37°C (Ο—Ο) over a 5-day period. Ordinate FPA values expressed as percentage of original activity and abscissa incubation time in days.
content of 0.05 ng/ml, which is below the range of 
detection in the FPA assay. Table 1 shows that 10 nl 
blood/ml urine was detected by Multistix and by FPA 
assay, while 1 nl blood/ml urine was barely detected 
by Multistix and not detected by FPA assay. The 
urine–blood mixtures in this experiment were not 
absorbed with bentonite; bentonite adsorbed the FPA 
reactivity consequent upon the addition of blood, leav-

ing only that present in the urine, 1.5 ng/ml.

**Urinary Excretion of FPA in Normal Individuals**

The mean 24-hr urinary excretion of FPA in 36 
males (ranging in age from 7–92) and 15 females (age 
range 12–59) was 1.67 μg, with a standard error of 0.1 
μg. The difference between male and female (1.71 ± 
0.2 μg/24 hr versus 1.56 ± 0.2 μg/24 hr) was not 
significant and there was no significant difference 
related to age, though children (n = 5) tended to 
exclude less FPA than adults (1.14 μg/24 hr versus 
1.73 μg/24 hr).

Plasma was obtained from 15 adults (mean age 
61 ± 13 yr) at the end of the urine collection period; 
plasma FPA was 1.26 ng ± 0.13 ng/ml. The urinary 
excretion in these 15 was 1.84 ± 0.47 μg/24 hr. 
Assuming a plasma volume of 2800 ml and a plasma 
half-life of FPA of 4 min, 635 μg FPA was cleaved 
from plasma fibrinogen/24 hr, and 1.84 μg or 0.29% of 
this total can be accounted for in the urine.

**Clearance of Human 125I-FPA From Dog**

Figure 2 (open circles) shows the plasma radioactiv-
ity determinations from a dog injected with 125I-FPA at 
0 time; the data are expressed as percent of the injected 
dose, assuming a plasma volume of 680 ml. At 0 time, 
50.3% of the injected radioactivity was present in the 
plasma, but there was a rapid fall over the first 5 min 
with a half-life of ~5 min; thereafter, there was a slow 
decline over the remainder of the observation period, 
with a half-life of ~90 min.

Plasma samples obtained over the first 12 min were 
chromatographed on a Sephadex G-25 column; Fig. 
3A shows the elution profile of 3 of the plasmas (2, 6, 
and 10 min), demonstrating that only part of the 
radioactivity is eluted as FPA. The proportion of 
radioactivity in the FPA-peak was determined for each 
sample by planimetry, and Fig. 2 (closed circles) shows 
the data for FPA in the plasma (expressed as percent 
of injected dose); the half-life was 3.9 min.

The dog's bladder was lavaged at the time of the 
injection. The urinary excretion, volume, and radioac-
tivity were recorded; after 3.5 hr, 9.3% of the injected 
radioactivity had been excreted in the urine. The urine 
samples were chromatographed on the Sephadex G-25 
column, and the sample with highest radioactivity is 
illustrated in Fig. 3B; it represents the collection period 
120–150 min after a saline infusion was started at 90 
min. The chromatographic elution patterns demon-
strate that only a small proportion of the urinary 
radioactivity was eluted in the FPA position, the major 
portion being eluted in lower molecular weight form. 
Total urinary FPA excretion over a 3.5-hr period was 
0.5% of the injected dose.

**Urinary Excretion of FPA in Patients**

Plasma samples were obtained from 14 patients with 
malignancy at the end of a 24-hr urine collection; the 
data are shown in Table 2 with patients' age and 
diagnosis. Plasma FPA is also shown from 3 patients in 
whom urine was obtained, but the data are omitted 
because of a positive reaction for blood. Only the 
urinary excretion is given for 5 patients, the plasma 
sample being discarded for technical reasons. Mean 
plasma FPA was 5.9 ng/ml and mean urinary FPA 
excretion was 8.7 μg/24 hr. Five patients had plasma 
FPA within the normal range and 7 had urinary FPA 
excretion within the normal range. Generally good 
correlation existed between plasma FPA and its urin-
ary excretion. Plasma and urine samples were also
Fig. 3. Sephadex G-25 chromatography of plasma and urine samples from a dog receiving an intravenous injection of human $^{125}$I-FPA at 0 min. The ordinates are radioactivity in counts/10 min and cpm and the abscissas is column effluent volume. (A) Results for 2 min (●), 6 min (●), and 10 min (△) plasma, and (B) those for the 120–150 min urine sample. $V_e$ for $^{125}$I-labeled FPA is shown on both diagrams. Note rapid diminution with time of the plasma FPA peak and increase of lower molecular weight radioactivity. 3B, similar in format to A, shows chromatography of the 120–150-min urine specimen. While a distinct activity peak is seen coincidental with the $V_e$ for $^{125}$I-FPA position, the majority of radioactivity is contained in another peak of lower molecular weight.

Table 2. Plasma FPA and Its Urinary Excretion in Patients With Malignancy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Plasma (ng FPA/ml)</th>
<th>Urinary Excretion (μg/24 hr)</th>
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</thead>
<tbody>
<tr>
<td>J.W.</td>
<td>Ca-lung</td>
<td>77</td>
<td>1.04</td>
<td>0.78</td>
</tr>
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<td>R.G.</td>
<td>Ca-lung</td>
<td>59</td>
<td>1.37</td>
<td>0.72</td>
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<tr>
<td>B.H.</td>
<td>Glioblastoma</td>
<td>60</td>
<td>1.56</td>
<td>10.5</td>
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<tr>
<td>K.W.</td>
<td>Ca-pancreas</td>
<td>62</td>
<td>1.88</td>
<td>0.92</td>
</tr>
<tr>
<td>C.R.</td>
<td>Ca-prostate</td>
<td>83</td>
<td>1.9</td>
<td>1.28</td>
</tr>
<tr>
<td>A.H.</td>
<td>Ca-prostate</td>
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<td>2.19</td>
<td>5.66</td>
</tr>
<tr>
<td>D.H.</td>
<td>Ca-lung</td>
<td>65</td>
<td>2.7</td>
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</tr>
<tr>
<td>H.H.</td>
<td>Ca-lung</td>
<td>58</td>
<td>3.6</td>
<td>5.5</td>
</tr>
<tr>
<td>M.E.</td>
<td>Ca-lung</td>
<td>67</td>
<td>4.1</td>
<td>3.65</td>
</tr>
<tr>
<td>W.W.</td>
<td>Ca-lung</td>
<td>56</td>
<td>4.5</td>
<td>3.7</td>
</tr>
<tr>
<td>A.M.</td>
<td>Ca-lung</td>
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<td>18.7</td>
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<td>P.P.</td>
<td>Ca-lung</td>
<td>73</td>
<td>7.1</td>
<td>19.8</td>
</tr>
<tr>
<td>R.C.</td>
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<td>57</td>
<td>15.6</td>
<td>24.8</td>
</tr>
<tr>
<td>W.H.</td>
<td>Ca-pancreas</td>
<td>71</td>
<td>17.2</td>
<td>32.6</td>
</tr>
<tr>
<td>S.J.</td>
<td>Ca-colon</td>
<td>68</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>J.M.</td>
<td>Ca-prostate</td>
<td>71</td>
<td>5.36</td>
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<tr>
<td>B.H.</td>
<td>Craniopharyngioma</td>
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<tr>
<td>H.H.</td>
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<td>1.22</td>
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<tr>
<td>A.C.</td>
<td>Ca-prostate</td>
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<tr>
<td>M.H.</td>
<td>Glioblastoma</td>
<td>60</td>
<td>20.4</td>
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</table>
obtained from two patients with alcoholic cirrhosis with mildly abnormal findings (~2.5 ng FPA/ml plasma and ~4.5 μg FPA/24 hr in the urine), and a patient with a cerebral abscess had a urinary excretion of 8.9 μg FPA/24 hr.

Twenty-four-hour urine collections were obtained from 7 patients with acute myocardial infarction over the first 5 days after admission to a coronary care unit. All samples were within the normal range in one patient, and 2 patients had a slight elevation in one sample each (day 1 and day 3, respectively), while greater elevations were seen in the remaining 4 patients, ranging from 5.35 to 34.4 μg/24 hr; 22 samples were assayed and the mean value was 5.35 ± 1.6 μg/24 hr, which differed from normal values (p < 0.01). In 3 of these patients there was a gross increase (2–7-fold) in FPA after thrombin treatment of the urine on day 1, though free FPA in 2 of them was within normal limits on that day; subsequent days showed higher than normal excretion of free FPA, with a smaller increase after thrombin treatment (mean 47%). Further analysis of two urine samples is shown in a later section.

Blood and urine samples were obtained from 2 severely burned patients over the first week following injury. Both were 20-yr-old men; R.C. had burns over 31% and B.Z. 81% of body surface area. Blood samples were obtained every day and sometimes more often; some samples were technically inadequate and were excluded from analysis. Some urine samples were positive for hemoglobin when tested by Multistix, but this phenomenon was due to hemoglobinuria. Figure 4 shows the data from plasma FPA determination (●) and urinary excretion of FPA (○); the points for urinary excretion are plotted at the end of each collection period.

Studies on three additional burn patients confirmed early elevation of plasma and urinary FPA and showed that these abnormalities persisted for several weeks.

**Thrombin Releasable FPA**

Twenty random urine samples from normal individuals as well as patients, concentration ranging from 1 to 20 ng FPA/ml, were assayed after pH adjustment with and without bentonite adsorption with virtually identical results (r = 0.99, y = 1.04x – 1.6). The addi-
tion of thrombin to normal urine samples resulted in slight increase of FPA, 19.6% ± 14% (SD). However, increase in assayable FPA was substantially greater in burn patients, 97% ± 53% (SD) and in those with malignancy, 47% ± 34% (SD). Paired plasma and urine samples from 2 burn patients (day 1 and day 5), from one patient with carcinoma of the prostate, and from a patient with carcinoma of the lung were dialyzed. The original plasma and urine samples (which had been kept at -70°C), the dialyzed plasma and urine, and the dialysates were assayed for FPA with and without thrombin treatment. The results are shown in Table 3 as the mean of all 4 studies.

The original plasma and urine showed respective increase in assayable FPA of 26% and 36% after thrombin treatment. Under the experimental conditions employed, 49% of plasma and 39% of urine FPA was found in the dialysate and respective increases of 29% and 9% of assayable FPA were observed on thrombin treatment of the dialysate. The dialyzed plasma and urines showed respective increases of 43% and 62% in assayable FPA after thrombin treatment. These findings demonstrated that both in bentonite-treated plasma and urine, fibrinogen fragments releasing FPA on thrombin treatment were present. A proportion of these were of sufficiently low molecular weight to be dialyzable.

Urine thrombin-releasable FPA was greatly increased in some of the patients with acute myocardial infarction, particularly early after disease onset. Figure 5A shows Sephadex G-25 chromatographic findings on a day 1 urine, FPA content 3.05 μg/24 hr, which on thrombin treatment showed a sevenfold increase in assayable FPA; the fibrinogen proteolysis products releasing FPA were eluted shortly after the column void volume. Figure 5B illustrates chromatographic findings in the day 2 urine sample from the same patient, when the FPA increase following thrombin treatment was 80%. Thrombin increasable FPA in these two samples (Fig. 5C) were calculated by subtracting the direct FPA assay values from those following thrombin treatment. The FPA containing fibrinogen breakdown products are of smaller molecular weight on day 2 than on day 1. Six other urine samples similarly analyzed showed findings more nearly resembling Fig. 5B than Fig. 5A.

**Correlation Between Plasma FPA and Its Urinary Excretion**

Figure 6 shows the data from all paired plasma–24-hr urine samples. There is significant correlation between plasma FPA and urinary excretion of FPA ($r = 0.80, n = 42, p < 0.001$), and the fully drawn line shows the relationship calculated from these data ($y = 1.10x + 4.89$). In the burn patients (shown as open circles), there is a lesser correlation between the two measures; in fact, when analyzed separately, the correlation is barely significant ($p < 0.05$). The correlation between plasma FPA and urinary excretion of FPA when the burn patients are excluded from the analysis is shown as a stippled line ($y = 1.85x + 0.09, r = 0.89, n = 28, p < 0.001$).

**DISCUSSION**

Mean urinary excretion in normal man was 1.67 ± 0.65 (SD) μg FPA/24 hr; which corresponds to 0.2%–0.5% of that calculated to be released into plasma over the same period of time. Roughly similar urinary excretion of radiolabeled human FPA was observed in the dog—0.5%.

Though urinary fibrinopeptide-A excretion is only approximately 0.5% of the total fibrinopeptide-A cal-
patients with malignancy (149) and 5 patients with severe burns samples from 15 normal individuals (shown as a square). The fully drawn line was calculated from all the data (y = 1.10x + 4.88, n = 42, r = 0.80, p < 0.001). The stippled line is calculated after exclusion of the burn patients (y = 1.85x + .09, n = 26, r = 0.89, p < 0.001).

Fig. 6. Plasma FPA versus urinary excretion of FPA in paired samples from 15 normal individuals (shown as a square), 16 patients with malignancy (■), and 5 patients with severe burns (○). The proteolysis products partially cross-reacted with FPA antiserum and are present in bentonite-adsorbed FPA (Fig. 3A), excluding the possibility, suggested by Wilner, that FPA was bound to a plasma protein.

The stability of FPA in urine (Fig. 1) was excellent at pH 5, while in the neutral range, more than half the reactivity was lost over a 5-day period. This finding is at variance with the stability studies of Qureshi and Nossel who found a greater loss over the first 24 hr, though their loss over 7 days was comparable to our 5-day loss. FPA instability in urine may have resulted in minor underestimation of FPA excretion, but this potential error, in the worst case, is probably less than 10% (Fig. 1). Urinary tract bleeding would give an artifactually high value for FPA excretion, however, tests with Multistix was of sufficient sensitivity to exclude significant bleeding (Table 1).

Urinary FPA, normally excreted in free form, was sometimes present in the form of fibrinogen proteolysis products, which release FPA on thrombin treatment. The proteolysis products partially cross-reacted with FPA antiserum and are present in bentonite-adsorbed plasma and urine. Two paired plasma–urine samples from burn patients and two from patients with malignancy, all with high plasma FPA, had, after dialysis, greater capacity to release FPA in the dialedyz sample than in the dialysate, with the difference being greater in urine than in plasma (Table 3), suggesting that the molecular weight of some of these breakdown products is greater than 12,000. A clear separation of fibrinogen breakdown products and free FPA was achieved with a urine sample (Fig. 5A, C), where the breakdown products were eluted in the same position as cytochrome-c (mol wt 12,400) on the same column (not shown), but this was close to the void volume. The data in Fig. 5A suggest that the reactivity of these fibrinogen breakdown products with FPA antiserum is approximately 10%.

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illustrated in Fig. 5A were not observed in six other urine samples with thrombin-releasable FPA. These latter samples, generally showing a lesser increase in FPA after thrombin treatment, had chromatographic findings similar to those in Fig. 5B. On the basis of elution position, molecular weight of these breakdown products were estimated to be ~2500, compatible with A-α-chain fragment 1-23 discussed by Nossel et al. as the most likely possibility in plasma. Four bentonite-adsorbed plasma samples showed similar fibrinogen moieties to those illustrated in Fig. 5B. These findings suggest that thrombin-releasable urinary FPA is a useful marker for states of systemic fibrinogenolysis.

Patients with acute myocardial infarction develop a coagulopathy, secondary to enhanced fibrin deposition, characterized by a several-fold increase in plasma high molecular weight fibrinogen complexes and lasting 10–18 days. They also have been reported to show early increase of plasma FPA, a mean value of 5 ng/ml during the first 4 days being shown in one study. We examined the urinary excretion of FPA in 7 myocardial infarction patients, and found greater than normal values on at least 1 day in 6 of them; mean and SE of 22 samples was 5.35 ± 1.63 ng FPA/24 hr (p < 0.01). An interesting finding was that of abnormally large thrombin-increasable FPA in three of these urines on the day of admission, even though free FPA was within the normal range in two of them on that day; these three patients on subsequent days had greater free FPA excretion than the other patients in the group.

Peuscher et al. studied 124 patients with a variety of malignancies and found normal plasma FPA values in 22% of patients, but a mean value of 5.5 ng/ml for the whole series. In the present study, 5 of 18 patients with malignancy had normal plasma FPA values, while the mean value for the group was 5.9 ng FPA/ml. Accepting normal urinary FPA excretion plus 2 SD as the upper limit of normal, 3.08 µg/24 hr, all but one of the patients with increased plasma FPA had increased urinary FPA excretion: The mean urinary FPA excretion in patients with malignancy was 8.7 µg/24 hr, which differs significantly from normal (p < 0.001).

Patients with severe burns have rapid fibrinogen turnover rates and evidence of intravascular fibrin formation with consumption of antithrombin-III. The present study shows high levels of plasma FPA and a correspondingly high urinary excretion; as in the earlier studies, these abnormalities persisted over several weeks, and Fig. 4 suggests reasonable correlation between the two measurements even with marked fluctuation of plasma FPA concentrations. However, correlation between plasma FPA and urinary excretion of FPA is not as close in the burn patients as it is in the other studies, p < 0.05—a finding probably explicable on the basis that plasma FPA defines thrombin action over a time period of minutes, while the urinary excretion of FPA covers a 24-hr period, as well as the previously mentioned tubular dysfunction.

Correlation between plasma FPA and its urinary excretion is significant (p < 0.001) for our combined patient data. Excluding the burn patients from the calculation, the relationship between plasma FPA (x) and its urinary excretion (y) is y = 1.85 x + .09, i.e., for each ng FPA/ml plasma, 1.85 µg FPA is expected to be excreted in the urine over 24 hr.

Our studies suggest that urinary excretion of FPA is a valid alternative measure of in vivo thrombin action. Assay of FPA 24-hr urinary excretion, rather than of FPA in venous blood as a measure of overall production, possesses the advantage that FPA values would be estimated over a long time period, whereas venous blood determinations provide data relevant only to a several minute time period. Yet, because of rapid FPA plasma clearance and rapid catabolism, both venous and urinary FPA determinations may not fully reflect FPA production since the site of specimen sampling is anatomically remote from the lesion responsible for FPA production. For instance, with lower limb deep venous thrombosis, blood must traverse the lungs, a likely major area of FPA catabolism, prior to reaching the kidney, and in addition, the upper extremity prior to venipuncture. In restricted circumstances, such as the patient with myocardial infarction, anatomic considerations might favor urinary over venous blood FPA studies, since the first pass of blood from the heart to the kidney does not require passage through the lungs, whereas arm venipuncture involves exposure of the blood to the pulmonary and extremity vasculatures.

Comparatively, little data are available on the variability of serial venous FPA studies in disease, and at least until this is shown to be reasonably small, determination of urinary FPA might usefully supplement venous blood studies. If venous serial FPA variability is large, urinary FPA assays might prove to be more representative of overall FPA production. Preliminary data suggest that shorter periods of urine collection, i.e., overnight, may be adequate for determining urine FPA excretion rates.

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