Serotonin, Catecholamines, Histamine, and Their Metabolites in Urine, Platelets, and Tumor Tissue of Patients with Carcinoid Tumors

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We monitored long-term (median 11 months) concentrations of platelet serotonin and urinary serotonin, 5-hydroxyindoleacetic acid, and seven catecholamine metabolites in 44 patients with carcinoid tumors. Tumor serotonin and catecholamine contents (11 patients) and urinary histamine and N-methylhistamine (15 patients) were determined. Consistently increased concentrations of indoles, notably platelet serotonin, were observed in 96%, 43%, and 0% of patients with mid-, fore-, and hindgut carcinoids, respectively. Urinary dopamine metabolites, notably 3-methoxytyramine, were consistently increased in 38%, 20%, and 7% of patients with mid-, hind-, and foregut carcinoids, respectively. For urinary norepinephrine/epinephrine metabolites, notably normetanephrine and metanephrine, these data were 33%, 20%, and 14%, respectively. Midgut carcinoid tumors had the highest serotonin contents, whereas concentrations of catecholamines were independent of primary localization. There was no consistent relation between biogenic amine contents in tumors and urinary excretion of the amine metabolites. Occurrence of carcinoid syndrome was related to increased serotonin production rate. Increased histamine production is not an important feature in patients with lung carcinoids or liver-metastasized ileum carcinoids.

Indexing Terms: tumor markers/cancer/5-hydroxyindoleacetic acid

Carcinoid tumors are endocrine neoplasms derived from enterochromaffin cells (1). They are usually classified according to their site of origin into carcinoids from foregut (respiratory tract, pancreas, stomach, and duodenum), midgut (ileum and appendix), and hindgut (left colon and rectum) (2). The endocrine manifestation of carcinoid tumors, referred to as the carcinoid syndrome, comprises flushing, diarrhea, valvular heart disease, and asthma-like symptoms (3, 4).

Because of their presumed embryonic origin from neuronal ectoderm and their ability to take up and decarboxylate amine precursors, Pearse (5) named intestinal carcinoids "gut APUDomas." The APUD (amine precursor uptake and decarboxylation) concept relates functional cell types to their ability to synthesize and store biogenic amines and polypeptides.5 Cells of the APUD system include pancreatic islet cells, pituitary cells, C-cells of the thyroid, gastrointestinal argentaffin cells, and chromaffin cells of the adrenal medulla (6). Tumors arising from these cell types, such as medullary carcinoma of the thyroid, carcinoid tumors, and pheochromocytoma, share a number of histological, ultrastructural, and biochemical properties.

Depending on their site of origin, carcinoid tumors can give rise to excessive synthesis, storage, and release of biogenic amines and polypeptide hormones (4, 7). Serotonin production is most prominent, notably in midgut carcinoids, and is related to the endocrine manifestations (1, 4, 7, 8). In addition, carcinoids have been reported to synthesize and secrete, in various proportions, 5-hydroxytryptophan [5-HTP (9–11)]; kallikrein (12); kinins (13); several neuropeptides, including substance P (14); prostaglandins (15); catecholamines (16); and histamine (17–19). From these substances, 5-HTP, catecholamines, histamine, bradykinin, and prostaglandins have been implicated in the etiology of the carcinoid syndrome symptoms (6).

On the basis of their confinement to the APUD system, additional production of catecholamines and histamine by carcinoid tumors is conceivable. Aromatic L-amino acid decarboxylase (EC 4.1.1.28) not only catalyzes decarboxylation of 5-HTP to serotonin, but also decarboxylates L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine (DA). Compared with 5-HTP decarboxylation, L-DOPA decarboxylation takes place at a similar Km and even higher Vmax (20). Aromatic L-amino acid decarboxylase also decarboxylates histidine but has a lower affinity for histidine than for 5-HTP and L-DOPA (21). Increased production of (notably) DA was found in patients with serotonin-producing carcinoid tumors (16). Studies on selected cases revealed the presence of DA, norepinephrine (NE), and epinephrine (E) in carcinoid tumor tissue from foregut and midgut (16, 22, 23). In addition, the catecholamine-synthesizing enzymes tyrosine hydroxylase, aromatic L-amino acid decarboxylase, and dopamine β-hydroxylase were found in an intestinal carcinoid tumor (24). Increased urinary histamine production was demonstrated in patients with gastric and ileal carcinoid tumors (18, 19).

We studied the frequency of increased urinary cate-

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5 Nonstandard abbreviations: APUD, amine precursor uptake and decarboxylation; 5-HT, 5-hydroxytryptophan; 5-HIAA, 5-hydroxyindoleacetic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; DA, dopamine; HVA, homovanillic acid; DOPAC, dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; NE, norepinephrine; E, epinephrine; VMA, vanillylmandelic acid; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol; NM, normetanephrine; and M, metanephrine.
Cholamine metabolite excretion during long-term monitoring of 44 consecutive carcinoid patients who were treated in our hospital over the last 5 years. The data were related to platelet serotonin content; urinary concentrations of 5-HIAA, serotonin, histamine, and N-methylhistamine; occurrence of the carcinoid syndrome; and the serotonin and catecholamine contents of the tumor tissue.

Patients and Methods

Patients

Forty-four consecutive patients (24 females, 20 males; median age 62, range 13–88 years) with histologically proven carcinoid tumors of fore-, mid-, and hindgut origin were studied (Table 1). Patients were monitored for as long as 5 years. Treatment included surgery, immunotherapy, or symptomatic therapy. The study protocol conformed to local ethical standards and the Helsinki declaration of 1975, as revised in 1983.

Samples

All samples were collected without dietary restrictions and in an undefined metabolic state. Venous blood was collected in 10-mL Vacutainer Tubes (Becton Dickinson, Meylan, France) containing 0.12 mL of 0.34 mol/L EDTA solution and put on ice without delay. Platelet-rich plasma was prepared within 1 h after sampling by centrifuging for 30 min at 120g and 4°C. Platelet concentrations were measured with a Coulter Counter Model S plus 4 (Coulter Electronics, Hialeah, FL). Na₂S₂O₅ and EDTA were added as preservatives to final concentrations of ~10 g/L each. Samples were analyzed within 1 week after collection.

Twenty-four-hour urines of patients were collected in 2-L brown polypropylene bottles (Sarstedt, Nürnberg, Germany), containing ~250 mg each of Na₂S₂O₅ and EDTA as preservatives. Samples were acidified to pH 4 with acetic acid and then frozen at −20°C. Samples were analyzed within 1 week after collection.

Tumor tissue from 11 patients was collected by biopsy or during surgical resections. Biopsies were necessary for histopathological diagnostic procedures. Samples were macroscopically characterized by histopathologists as carcinoid tissue. Confirmation of the carcinoid aspect of tumor tissue was obtained by histopathological examination. One part was immediately transferred to a 0.01 mol/L acetic acid solution containing 10 g/L each of Na₂S₂O₅ and EDTA. Tissue samples were homogenized (Potter S homogenizer; Braun, Melsungen, Germany) at 0°C in 10 mL of a 0.01 mol/L acetic acid solution containing Na₂S₂O₅ and EDTA at final concentrations of 100 g/L each. After homogenization, samples were stored at −20°C. Analysis was performed within 1 month after collection.

Analytical Methods

Serotonin and metabolites. Serotonin in platelet-rich plasma, urine, and supernates of tissue homogenates was determined by HPLC with fluorometric detection, as described by Kwarts et al. (25). Platelet serotonin content, expressed in nmol/10⁸ platelets, was calculated by dividing the concentration of serotonin in platelet-rich plasma by the concentration of platelets in platelet-rich plasma. Urinary 5-HIAA was determined in ether extracts by HPLC with fluorometric detection, essentially as described by Rosano et al. (26).

Catecholamines and metabolites. Urinary acidic (homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), vanillylmandelic acid (VMA)) and alcoholic [3-methoxy-4-hydroxyphenylethylglycol (MHPG)] catecholamine metabolites were determined in enzymatically hydrolyzed urine samples, with use of capillary gas chromatography with flame ionization detection, according to our previously described method (27).

Urinary total 3-O-methylated catecholamine metabolites [3-methoxytyramine (3MT), normetanephrine (NM), and metanephrine (M)] were determined in alkaline ethyl acetate extracts by stable isotope mass fragmentography (28).

Catecholamines were isolated from tissue homogenates by the paired-ion-extraction method of Smedes et al. (29). 3,4-Dihydroxybenzylamine served as an internal standard. Catecholamine (DA, NE, and E) contents of tissue homogenates were determined by HPLC with electrochemical detection as previously described (30).

Table 1. Clinical characteristics of 44 patients with carcinoid tumors of various origins.

<table>
<thead>
<tr>
<th>(female, male)</th>
<th>Age, years a</th>
<th>Location of primary tumor b</th>
<th>Metastases b</th>
<th>Carcinoid syndrome b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foregut</td>
<td>14 (5, 9)</td>
<td>Lung (12), epiglottis (1), larynx (1)</td>
<td>None (7), lymph nodes (4), multiple sites (3), bone (2), brain (2), lung (1), skin (1)</td>
<td>Flushing (1), diarrhea (1), none (12)</td>
</tr>
<tr>
<td>Midgut</td>
<td>25 (18, 7)</td>
<td>Ileum (21), appendix (1), Meckel's diverticulum (1), ovary (1), unknown (1)</td>
<td>Liver (19), multiple sites (3), lymph nodes (2), retroperitoneal (2), intraperitoneal (1), supracavicular (1), bone (1), unknown (2)</td>
<td>Flushing (19), diarrhea (19), heart disease (3), asthma (3), none (2)</td>
</tr>
<tr>
<td>Hindgut</td>
<td>5 (1, 4)</td>
<td>Rectum (5)</td>
<td>Liver (4), skin (1), none (1), multiple sites (1)</td>
<td>None (5)</td>
</tr>
</tbody>
</table>

a Median and ranges. 
b Numbers in parentheses denote number of patients.
Histamine, N-methylhistamine, and creatinine. Urinary histamine and N-methylhistamine were determined by isotope dilution mass fragmentography, according to Keyzer et al. (31, 32). Urinary creatinine contents, used to express analyte concentrations in terms of urinary excretion rate, were measured by a picric acid method with an SMA-2 analyzer (Miles Technicon Instruments, Tarrytown, NY).

Data Analysis and Statistics

Calculations of medians and ranges for analyte concentrations during long-term monitoring were performed as follows. For each patient all available data of a single analyte were averaged. To minimize the effects of therapeutic intervention, we omitted results from the moment that treatment caused pretherapeutically increased platelet serotonin (the most sensitive marker, see below) to reach normal values. From the average analyte concentrations of individual patients we calculated the median and range for each of the carcinoid patient subgroups. The significance of differences between subgroups of patients was calculated with the Kruskal–Wallis one-way analysis of variance (33). \( P < 0.025 \), adjusted for type I errors according to Holm (34), were considered significant.

To estimate the sensitivity of these analytes for carcinoid tumors, we used the upper limits of previously reported reference ranges for platelet serotonin and for urinary 5-HIAA, serotonin (8), HVA, DOPAC, VMA, MHPG (35, 36), 3-MT, NM, and M (28). Evaluation was based on two criteria. To qualify as having increased concentrations of a single analyte according to the first criterion, a patient had to exhibit at least one value above the upper limit of the reference range when only two data points were available, or at least two above-normal values when more data were available. To qualify as having increased values according to the second criterion, a patient had to exhibit consistently increased concentrations for that analyte. Concentrations of analyte groups—"indoles" (platelet serotonin; urinary 5-HIAA and serotonin), "DA metabolites" (urinary HVA, DOPAC, and 3-MT), and "NE/E metabolites" (urinary VMA, MHPG, NM, and M)—were considered increased when at least one of the analyte members of the group met the above criteria.

Results

Clinical characteristics. Table 1 lists the clinical characteristics of the study group of 44 carcinoid patients. Patients were classified according to the site of tumor origin. Most (86%) of the 14 patients with foregut carcinoid tumors had primary tumors in the lung, without symptoms of the carcinoid syndrome. Two patients with long-lasting disease had multiple metastases, which coincided with symptoms of the carcinoid syndrome. In the group of 25 midgut carcinoid patients, the ileum was the major primary site; metastases were mainly located in the liver. Except for two (one ovarian and one ileum carcinoid), all patients with metastasized midgut carcinoids had one or more symptoms of the carcinoid syndrome. None of the five patients with hindgut carcinoids presented with symptoms of the carcinoid syndrome.

Serotonin, catecholamines, and metabolites in platelets and urine. Table 2 shows platelet serotonin contents and urinary concentrations of 5-HIAA, serotonin, and seven catecholamine metabolites for the study group of 44 carcinoid patients, together with the upper limits of the respective reference ranges. For indoles, the data were calculated from a median number of 6 assays (range 1–55) per patient; for catecholamines, the median number of assays per patient was 4 (range 1–35). The median follow-up period per patient was 11 months (range 1 day–5 years). Omission of data because of treatment—surgical debulking or complete tumor removal—was

| Table 2. Platelet serotonin and urinary 5-HIAA, serotonin, and catecholamine metabolites of patients with carcinoid tumors. Median conc (and range)* |
|----------------------------------|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|          |                               |          |          |          |          |          |
| Plateletb | Urine*                        | 5-HIAA* | DA metabolites | NE/E metabolites |
|          |                               |          | HVA* | DOPAC* | 3-MT* | VMA* | MHPG* | NM* | M*                  |
| Foregut (n = 14) |                               |          |          |          |          |          |          |          |          |
| 5.1±3  | 2.7                            | 1.8     | 0.9     | 1.9     | 1.1    | 154    | 66     |
| (2.0–18.4) | (1.8–6.1)                     | (0.4–1.3) | (90–234) | (10.0–2.3) | (0.5–1.4) | (67–330) | (42–171) |
| Midgut (n = 25) |                               |          |          |          |          |          |          |          |          |
| 29.7±3  | 3.6                            | 27.7±6  | 0.9     | 1.7     | 0.9    | 232    | 60     |
| (5.4–44.7) | (1.9–12.7)                    | (0.3–3.8) | (43–1488) | (0.9–3.9) | (0.4–2.6) | (69–877) | (34–274) |
| Hindgut (n = 5) |                               |          |          |          |          |          |          |          |          |
| 1.7±3   | 2.3                            | 2.0±6   | 0.5     | 1.9     | 1.3    | 239    | 68     |
| (0.7–6.4) | (2.2–3.0)                     | (0.4–0.6) | (86–260) | (1.6–2.4) | (1.0–1.9) | (130–357) | (62–110) |
| Reference* |                               |          |          |          |          |          |          |          |          |
|          | 5.4                            | 3.8     | 5.5     | 2.0     | 170    | 2.5    | 1.5   | 280  | 70                  |

* Matching superscript numbers denote statistically significant differences (Kruskal–Wallis, \( P < 0.025 \)).
* mmol/10³ platelets.
* μmol/mol creatinine.
* mmol/mol creatinine.
* Upper limits of respective reference ranges.
necessary in three cases. Immuno- and systemic therapy was found to affect serotonin and catecholamine concentrations (37). The effects were, however, not such that they led to long-term reduction of marker concentrations below the upper limits of the reference values, as was found for the three surgically treated patients. Fig. 1 shows the concentrations of platelet serotonin, urinary serotonin, HVA, DOPAC, 3-MT, VMA, MHPG, NM, and M for 10 selected patients with carcinoid tumors. Selection was based on increased urinary excretion of one or more catecholamine metabolites during follow-up.

Platelet serotonin contents differed significantly between the three subgroups, with the highest values in the patients with midgut carcinoids and the lowest in those with hindgut carcinoids (Table 2). Urinary 5-HIAA of patients with midgut carcinoids was significantly higher than that of patients with fore- and hindgut carcinoids. Urinary serotonin excretion was greatest in midgut carcinoid patients, and differed significantly between fore- and midgut carcinoid patients. Although median values and upper limits of urinary DA-metabolite excretion were highest in midgut carcinoid patients, no significant differences were found between the three subgroups. Subgroup differences between urinary excretion of NE and E metabolites were not significant either.

Figure 2 shows an example of long-term monitoring of platelet serotonin and of urinary 5-HIAA, serotonin, and catecholamine metabolites in a patient with an ileum carcinoid and liver metastases. In December 1987 this patient underwent resection of the primary tumor and a large liver metastasis in the left lobe. The liver metastasis contained high concentrations of serotonin and DA (sample 8, Table 3). A small metastasis was left in situ in the right lobe. The postoperative platelet serotonin content was significantly reduced but remained above the upper limit of the reference range during the postoperative observation period. Urinary excretion of 5-HIAA and serotonin was also reduced markedly after

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**Fig. 1.** Concentrations of indoles (platelet serotonin content, urinary 5-HIAA, serotonin), urinary dopamine metabolites (HVA, DOPAC, 3-MT), and urinary NE and E metabolites (VMA, MHPG, NM, M) for 10 patients with carcinoid tumors of midgut (1-8), foregut (9), and hindgut (10) origin. Selection of patients was based on increased urinary excretion of one or more catecholamine metabolites during follow-up. Horizontal bars indicate average values, dotted lines indicate the upper limits of the reference ranges. Note the logarithmic ordinate for 5-HIAA. PLT, platelet; 5-HT, serotonin.
operation and transiently stabilized at and below the upper limits of reference ranges, respectively. With the surgery, urinary concentrations of DA and NE/E metabolites dropped to normal values. With gradual progressive disease, however, platelet serotonin and to a lesser extent urinary 5-HIAA increased. Urinary serotonin showed a less consistent pattern. From the urinary catecholamine metabolites, only DOPAC gradually increased, but this increase took place within the normal reference range.

Urinary histamine and N-methylhistamine. Urinary histamine and N-methylhistamine concentrations were examined in 15 patients (5 males, 10 females; primary locations: six foregut, eight midgut, and one hindgut). For each patient an average of 5 urine samples (range 1–18) was examined. In one patient with an ileum carcinoid and hepatic metastases, 1 of 18 determinations indicated a highly increased urinary histamine value [532 µmol/mol creatinine; upper limit of reference range 75 (31)]. However, her N-methylhistamine was within the reference range. Two midgut carcinoid patients, each examined once, exhibited borderline increased urinary N-methylhistamine [155 and 207 µmol/mol creatinine; upper limit of reference range 150 (32)].

Carcinoid sensitivity of serotonin and catecholamines. Table 4 shows the estimated sensitivities of serotonin and catecholamines as markers for carcinoid patients during a prolonged observation period. To investigate the consistency of increased concentrations of a marker, we calculated sensitivity in two ways. By the first criterion, the data represent the percentage of patients who exhibited at least one increased value in cases with

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**Table 3. Serotonin and catecholamine contents of tumor biopsies from patients with carcinoid tumors**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serotonin, nmol/g wet tissue</th>
<th>DA, pmol/g wet tissue</th>
<th>NE, pmol/g wet tissue</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>52 680</td>
<td>6540</td>
<td>n.d.</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>31</td>
<td>52</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>0.02</td>
<td>195</td>
<td>15 030</td>
<td>83</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12 500</td>
<td>13 700</td>
<td>4800</td>
<td>106</td>
</tr>
<tr>
<td>5</td>
<td>1200</td>
<td>4000</td>
<td>210</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>370</td>
<td>390</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>36 400</td>
<td>2180</td>
<td>750</td>
<td>1685</td>
</tr>
<tr>
<td>8</td>
<td>28 400</td>
<td>8700</td>
<td>73</td>
<td>n.d.</td>
</tr>
<tr>
<td>9</td>
<td>4420</td>
<td>3600</td>
<td>240</td>
<td>n.d.</td>
</tr>
<tr>
<td>10</td>
<td>180</td>
<td>550</td>
<td>15 000</td>
<td>n.d.</td>
</tr>
<tr>
<td>11</td>
<td>0.03</td>
<td>n.d.</td>
<td>45</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* Tissue from primary tumors and metastases was obtained from three foregut carcinoids (samples 1–3), seven midgut carcinoids (4–10), and one hindgut carcinoid (11).

n.d., not detectable.
Table 4. Sensitivities of platelet (PTL) serotonin (5-HT) and urinary 5-HIAA, serotonin, and catecholamine metabolites for patients with carcinoid tumors.

<table>
<thead>
<tr>
<th>% of patients with above-normal analyte*</th>
<th>Foregut</th>
<th>Midgut</th>
<th>Hindgut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indolesb</td>
<td>57/43 (14)</td>
<td>100/96 (25)</td>
<td>60/0 (5)</td>
</tr>
<tr>
<td>PTL 5-HT</td>
<td>50/43 (14)</td>
<td>100/96 (25)</td>
<td>20/0 (5)</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>29/14 (14)</td>
<td>92/76 (25)</td>
<td>0/0 (5)</td>
</tr>
<tr>
<td>5-HT</td>
<td>55/18 (11)</td>
<td>82/46 (22)</td>
<td>60/0 (5)</td>
</tr>
<tr>
<td>DA metabolitesb</td>
<td>25/7 (12)</td>
<td>63/38 (24)</td>
<td>20/20 (5)</td>
</tr>
<tr>
<td>HVA</td>
<td>8/0 (12)</td>
<td>42/4 (24)</td>
<td>0/0 (5)</td>
</tr>
<tr>
<td>DOPAC</td>
<td>0/0 (12)</td>
<td>17/13 (24)</td>
<td>0/0 (4)</td>
</tr>
<tr>
<td>3-MT</td>
<td>20/10 (10)</td>
<td>65/35 (17)</td>
<td>25/25 (4)</td>
</tr>
<tr>
<td>NE/E metabolitesb</td>
<td>33/14 (12)</td>
<td>50/33 (24)</td>
<td>60/20 (5)</td>
</tr>
<tr>
<td>VMA</td>
<td>8/0 (12)</td>
<td>21/8 (24)</td>
<td>20/0 (5)</td>
</tr>
<tr>
<td>MHPG</td>
<td>9/0 (11)</td>
<td>29/8 (24)</td>
<td>50/0 (4)</td>
</tr>
<tr>
<td>NM</td>
<td>20/20 (10)</td>
<td>30/12 (17)</td>
<td>50/0 (4)</td>
</tr>
<tr>
<td>M</td>
<td>40/20 (10)</td>
<td>47/24 (17)</td>
<td>50/25 (4)</td>
</tr>
</tbody>
</table>

* Expressed as % by criterion 1% by criterion 2 (and no. of patients evaluated). To qualify as having increased values of a single analyte according to the first criterion, a patient had to exhibit at least one value above the upper limit of the reference range in the case of two available data, or at least two above-normal values when more data were available. To qualify as having increased values according to the second criterion, a patient had to exhibit consistently increased values for that analyte.

b Analyte group values were considered increased when at least one of the analyte members of the group met the criteria given above. Occasionally, sensitivities of single analytes were greater than those of analyte groups because of differences in number of patients evaluated.

two available results and two or more increased values when more data were available. This criterion proved comparable with at least 20% of the values being increased in a series of measurements in one patient. According to the second criterion, the data represent the percentage of patients who had increased values for that analyte in all analyzed samples.

The results show that platelet and urinary indoles are the most sensitive of these analytes for patients with midgut and foregut carcinoid tumors. Consistently increased amounts of indoles, notably platelet serotonin, were observed in 98% and 43% of patients with midgut and foregut carcinoids, respectively. None of the patients with hindgut tumors showed consistently increased platelet serotonin or urinary indoles. Evaluation of the data by both criteria showed that urinary DA and NE/E metabolites are also frequently increased, notably in patients with midgut carcinoids (in 38% and 33% of these patients, respectively). Urinary 3-O-methylated catecholamine metabolites (3-MT, NM, and M) showed the highest sensitivities of all the catecholamine metabolites. Depending on the location of the primary tumor, urinary 3-MT, NM, and M showed consistently increased values in 10–35%, 0–20%, and 20–25%, respectively, of all patients with carcinoid tumors.

Tumor serotonin and catecholamine contents and their relation to platelet and urinary values. Table 3 shows serotonin and catecholamine contents in tumor tissue samples from 11 carcinoid patients. In seven tumor samples DA was the most abundant catecholamine, whereas NE predominated in the remaining four. High tumor serotonin contents frequently coincided with increased mean concentrations of platelet serotonin and urinary 5-HIAA (data not shown). However, neither tumor serotonin nor catecholamine contents were consistently correlated with platelet serotonin and urinary 5-HIAA, or urinary catecholamine metabolite concentrations, respectively. High catecholamine contents in tumors coincided with above-normal urinary catecholamine metabolites in five patients (Table 3; nos. 5, 6, 8, 9, and 10). On the other hand, normal urinary catecholamines were found in four patients with high tumor catecholamine contents (nos. 1, 3, 4, and 7). The patient with liver-metastasized hindgut carcinoid (no. 11) had a relatively low tumor catecholamine content, but consistently presented with moderately increased urinary catecholamine metabolites.

Relation between biogenic amines and carcinoid syndrome. Except for two patients with normal urinary 5-HIAA, all 25 patients (Table 1) who exhibited symptoms of the carcinoid syndrome had moderately to highly increased concentrations of platelet serotonin and urinary 5-HIAA. On the other hand, seven patients with borderline or moderately increased platelet serotonin (all but one with normal urinary 5-HIAA excretions) did not show any signs of the carcinoid syndrome. Thirteen of the patients with carcinoid syndrome had increased urinary excretion of DA metabolites, and seven had increased excretion of both DA and NE/E metabolites. None of the patients with carcinoid syndrome had increased catecholamine metabolites without additionally increased indoles. Of the 20 patients who exhibited flushing, 12 had increased platelet serotonin and increased urinary excretion of catecholamine metabolites. None of the patients who exhibited flushing had increased catecholamine metabolites without additionally increased indoles. The three patients with occasionally increased urinary excretion of histamine or N-methylhistamine showed symptoms of the carcinoid syndrome, including flushing. All three had additionally increased platelet serotonin, urinary indoles, and urinary catecholamine metabolites.

Discussion

Indoles

Estimation of the sensitivities of indoles in 44 patients with carcinoid tumors (Table 4) shows that, of all analytes, platelet serotonin is the most sensitive and consistently increased marker during long-term monitoring, notably for tumors originating from fore- and midgut. Data from the small number of patients with hindgut carcinoids suggest that serotonin production by these tumors is not only lower (Table 2) but also less consistent during long-term follow-up (Table 4). For the clinical chemical diagnosis of hindgut carcinoids, it seems appropriate to repeat indole analyses over an extended period. These findings confirm and extend our previous report on pretherapeutic results for 30 patients from the present study group (8). For the sake of simplicity we consistently used the term platelet serotonin in this study to refer to the serotonin concentration in
platelet-rich plasma. The correct term, however, should be “apparent platelet serotonin” because, as we showed in carcinoid patients with increased serotonin production rate, the serotonin in platelet-rich plasma is not necessarily confined to platelets.

**Urinary Catecholamines**

Results from urinary catecholamine metabolite analyses (Tables 2 and 4, Fig. 1) indicate that catecholamine production is frequently increased in patients with carcinoids, especially in those with midgut carcinoids. The number of reports on this subject is small (16, 22-24, 38-40). In a study of 64 biopsy-proven carcinoid patients, Feldman (16) measured urinary excretion of free catecholamines, HVA, and plasma catecholamines. In the 45 patients with serotonin-secreting carcinoid tumors, he found that 27%, 15%, and 0% had increased urinary free DA, NE, and E, respectively; 18% had increased excretion of HVA, and in 5 of 14 patients (35%) plasma DA was increased. The 19 patients with non-serotonin-producing carcinoid tumors did not show evidence of excessive DA production. On the other hand, increased urinary free NE, but not E, was observed in patients with both serotonin-secreting and -nonsecreting carcinoids and in patients with miscellaneous tumors.

The present study confirms frequently increased urinary DA metabolites and less frequently increased NE/E metabolites in carcinoid patients. However, as shown in Fig. 1, we also found increased urinary catecholamine metabolites (originating from both DA and NE/E) in two patients with non-serotonin-producing carcinoids (nos. 9 and 10, Fig. 1). For the urinary excretion of catecholamine metabolites during follow-up, our data for sensitivities indicate that, in patients with fore-and midgut carcinoids, increased production of catecholamines is less consistent than that of indoles (Table 4). Urinary 3-O-methylated catecholamine metabolites showed the highest sensitivity and consistency of all catecholamine metabolites in the three patient subgroups. Because these metabolites predominantly derive from deactivation of circulating catecholamines by catechol-O-methyltransferase (EC 2.1.1.6) (41), this indicates that carcinoid tumors can give rise to increased concentrations of circulating free catecholamines. The moderate increase in urinary catecholamine metabolites, notably NM and M (42-44), in patients with carcinoid tumors may give rise to confusion in the laboratory diagnosis of catecholamine-secreting tumors such as pheochromocytoma.

**Catecholamines in Carcinoid Tumors**

Increased urinary concentrations of catecholamines in patients with carcinoid tumors can be attributed to several factors. Diet, drugs, activity, and stress modify both relative proportions and absolute excretion amounts of catecholamine metabolites (41). As previously noted (16), increased urinary free catecholamines in carcinoid patients may be caused by malignant tumor-related psychological and physical stress with concomitant activation of the sympathetic nervous system. Stress may certainly contribute to increased urinary catecholamine (metabolite) excretion (notably NE, E, and their metabolites) in carcinoid patients, but catecholamine production by the tumor (notably DA) can not be excluded (see below). The follow-up of the patient presented in Fig. 2 suggests that tumor-derived catecholamines may significantly contribute to increased urinary catecholamine metabolite excretion, although other explanations are also possible. Reduction of tumor mass coincided with normalization of urinary catecholamine metabolites. The tumor (no. 8 in Table 3) was found to contain substantial amounts of DA, and subsequent growth of residual tumor coincided with gradually increasing urinary DOPAC excretion.

High catecholamine contents in carcinoid tumors (Table 3) may originate from partial de novo synthesis, uptake from the circulation, de novo synthesis, or combinations of these. Given that carcinoid tumors are APUDomas, uptake of circulating L-DOPA is conceivable and may cause subsequent synthesis of DA. The responsible enzyme, aromatic L-amino acid decarboxylase, is at least present in all carcinoid tumors that are characterized by serotonin production. Enterochromaffin cell-associated submucosal neurons (45) and platelets (46, 47) have the ability to take up circulating serotonin and catecholamines. Platelets possess uptake systems for serotonin and to a lesser extent catecholamines (47, 48). Consequently, uptake of catecholamines from the circulation may occur in neoplastic counterparts of carcinoid tumors that possess a serotonin reuptake system. Demonstration of tyrosine hydroxylase, aromatic L-amino acid decarboxylase, and dopamine β-hydroxylase in carcinoid tumors (24, 16) suggests that both de novo synthesis of DA and NE from tyrosine and partial de novo synthesis from accumulated DA from extracellular sources are possible. No data are available on the presence of phenylethanolamine-N-methyltransferase in carcinoid tumor tissue. It is therefore unclear whether the repeated demonstration of E in carcinoid tumor tissue (22, 23, 49) is caused by uptake or (partial) de novo synthesis.

The catecholamine contents of carcinoid tumor tissue are the result not only of uptake and synthesis, but also of storage capacity, catabolizing activity, and frequency of release. Differences in the relative magnitudes of these factors and interindividual differences in tumor burden may explain the lack of relationship between tumor amine content and urinary metabolite concentrations. We found that neither tumor serotonin nor tumor catecholamine contents were consistently related to platelet serotonin and urinary 5-HIIAA or to concentrations of urinary catecholamine metabolites. Because this discrepancy was most apparent for catecholamines, we suggest that secretion of serotonin and secretion of catecholamines from carcinoid tumors do not necessarily coincide. The discrepancy may be attributable to storage in different cell types or distinct secretory granules within a single cell type (23, 24).
**Histamine**

Urine samples from patients with 5-HTP-secreting foregut (gastric) carcinoids and patients with ileum carcinoids who exhibited the carcinoid syndrome were found to contain increased concentrations of histamine (18, 19). Of 10 patients with midgut (ileum) carcinoids and carcinoid syndrome symptoms, 2 had normal urinary histamine excretion, 4 slightly increased excretion, and 4 highly increased excretion (19). Snow (18) found large quantities of histamine in a gastric carcinoid tumor. Circumstantial evidence for histamine production by gastric carcinoids was derived from the observation that the typical flush in patients with gastric carcinoid tumors could be prevented by histamine H₁ and H₂ receptor antagonists (18, 50). However, studying both urinary histamine and N-methylhistamine contents in our patient group, we found no consistent proof of excessive histamine production. A probable explanation for the discrepancy is that our study did not include patients with gastric carcinoids. However, the early observation of Gowenlock and Platt (19), that histamine production also occurs frequently in midgut carcinoids with carcinoid syndrome, is not confirmed by our data, although discrepancies attributable to different patient cohorts cannot be ruled out. Measurement of urinary histamine should be considered an unreliable index for estimating the rate of histamine production by the human body. In contrast to its metabolite N-methylhistamine, urinary histamine may give rise to both false-positive and false-negative results. False-positive results may be caused by bacterial histamine production, notably in the urogenital tract and after urine collection, whereas five of eight patients with mastocytosis showed normal urinary histamine contents (51). We found only two slightly increased concentrations of urinary N-methylhistamine in our patient group; thus, we conclude that, in patients with lung carcinoids or ileum carcinoids with liver metastases, excessive histamine production is not an important feature.

**Carcinoid Syndrome**

Release of catecholamines from carcinoid tumors may be of clinical importance: intravenous administration of 6–60 nmol of NE and 3–16 nmol of E have been shown to cause flushing in serotonin-producing carcinoid patients within 2 min (52, 53). Catecholamines may be released, e.g., in patients with pheochromocytoma. However, the administered dose caused the typical carcinoid flush only in carcinoid patients (52). This suggests that, in carcinoid patients, catecholamines cannot be the sole mediators of flushing but are able to initiate a cascade that results in flushing. Experiments with the pentagastrin provocation test gave insight into the mechanism of catecholamine-induced flushing in carcinoid patients (54–56). In patients with midgut carcinoids and hepatic metastases, the pentagastrin provocation test causes flushing after the release of serotonin in excess of the patients' metabolizing capacity (55). This process has been shown to proceed indirectly via pentagastrin-induced release of catecholamines from the adrenals (54, 55); in turn, the released catecholamines activate β-adrenoreceptors on carcinoid tumor cells, leading to serotonin release (56). This process is analogous to the catecholamine-induced serotonin release from gut enterochromaffin cells, which is also controlled by β-adrenoreceptors (56). The data in Table 3 show that sudden massive catecholamine (notably NE) release from carcinoid tumors may result in circulating concentrations comparable with those of the intravenous injection experiments (52, 53). Moreover, catecholamine release from tumor cells will produce higher local concentrations than intravenous injection. Local release of catecholamines within a carcinoid tumor may initiate a chain reaction that results in a massive release of tumor serotonin. Because carcinoid tumor cells contain not only serotonin, but also other tentative mediators of the carcinoid syndrome symptoms (4), release of these compounds may also contribute to subsequent events. Although concentrations of catecholamines in carcinoid tumors are generally lower than those found in pheochromocytoma tumor tissue, increased circulating concentrations of catecholamines may also cause systemic effects.

In conclusion, platelet serotonin is the most sensitive and consistently increased marker during long-term monitoring of patients with carcinoid tumors, notably those with tumors originating from fore- and midgut. Analyses of urinary catecholamine metabolites indicate that increased catecholamine production by patients with carcinoid tumors occurs frequently, especially in those with midgut carcinoids. DA metabolites were more frequently increased than NE/E metabolites. Urinary 3-O-methylated catecholamine metabolites showed the highest sensitivity and consistency of all catecholamine metabolites, indicating an increase in circulating catecholamines. Malignant tumor-related stress may play a role in increased production of NE and E metabolites but not of DA metabolites. Increased urinary NE and E metabolites may render differentiation with the diagnosis of pheochromocytoma difficult. It is as yet unclear whether catecholamines in carcinoid tumors originate from de novo synthesis, partial de novo synthesis, uptake from the circulation, or some combination of these. Release of tumor catecholamines may be involved in the initiation of carcinoid flushing. Differences in tumor amine storage capacity, catabolizing activity, and frequency of release may explain the lack of relationship between tumor amine content and urinary metabolite concentrations. Excessive histamine production is not an important feature in patients with lung carcinoids or liver-metastasized ileum carcinoids.

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