Total Urinary Catechol Excretion in Cardiovascular and Other Clinical Conditions

By Wilhelm Raab, M.D. and Wilda Gigee, A.B.

Although colorimetry (method of Shaw) permits neither a differentiation of epinephrine and norepinephrine nor their separation from other catechols, it reveals the total urinary catechol excretion, including those portions which were inactivated in the body. No significant abnormalities were found in arterial hypertension, in congestive heart failure, after myocardial infarction and during emotional stress. Thoracolumbar sympathectomy depressed total catechol excretion temporarily. In renal uremia, the regularly demonstrable elevation of the blood catechols was paralleled by a diminution of the conjugated catechols in the urine. It appears to be due to renal retention.

The increasing appreciation of the pathogenetic importance of sympathoadrenal neurosecretion in cardiovascular pathology has prompted various attempts to gauge the state of function of the sympathetic nervous system and of the adrenal medulla by means of assay of epinephrine, norepinephrine and related compounds in the urine. Holtz and co-workers detected increased amounts of pharmacodynamically active catecholamines in the urine of 16 out of 23 patients with arterial hypertension. This was confirmed by Kroneberg and Schümann but Goldenberg and Rapport recorded abnormally high values in only 2 out of 14 patients with essential hypertension.

The colorimetric test of Shaw permits the isolation and recovery of all chromogenic catechol compounds. It reveals the presence in the urine of chromogenic material far in excess of what can be assumed to be the combined amounts of active epinephrine, norepinephrine and hydroxytyramine. In addition, acid hydrolysis intensifies the color reaction markedly. It has been concluded, therefore, that the urine contains, beside the active material, considerable quantities of both free and conjugated derivatives of deaminated epinephrine and norepinephrine with an intact catechol nucleus.

Using the colorimetric assay of total catechols in the nonhydrolyzed and hydrolyzed urines of normal and hypertensive individuals, Kroneberg and Schümann did not observe any difference between the two groups regarding the nonconjugated chromogenic catechols but the conjugated catechols, as recovered by hydrolysis, appeared to be markedly augmented in the hypertensive group (average +73 per cent). Nuzum and Bischoff did not confirm the latter finding. In their series, no significant difference was apparent in the urines of normal and hypertensive subjects. Only in three patients with myocardial infarction did they observe an increased ratio of the readings from hydrolyzed and nonhydrolyzed urine.

Shaw's so-called "specificity test" (color ratio of alkali- and acid-treated specimens), upon which Nuzum and Bischoff based their calculation of the absolute amounts of excreted epinephrine, is not suitable for the differentiation of epinephrine and norepinephrine since it applies to both substances. Moreover, the difference in color intensity yielded by epinephrine and norepinephrine (4.4 parts norepinephrine equalling 1 part epinephrine), and the uncertainty regarding the degree of chromogenicity of the other participating (deaminated?) catechol compounds in the urine impair the conclusiveness of the "specificity" ratio.

In the following, we shall report the total color readings obtained in nonhydrolyzed and

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hydrolyzed urine specimens of normal individuals and of patients with cardiovascular diseases and other clinical conditions.

**METHODS**

In most instances, the urine specimens were collected over 12-hour periods (from 7 p.m. to 7 a.m.) in vessels which contained sodium bisulphite (about 10 per cent) for stabilization of the catecholamines. Following the procedure described by Kroneberg and Schümann,2 the urine volumes were brought up to 1000 cc. with distilled water. Ten cc. of the diluted urine were again diluted with 80 cc. of distilled water. Two cc. of this second dilution were placed in each of two test tubes for further analysis with the modified method of Shaw, as described in detail elsewhere,19 using epinephrine standards which were kept constant with a Coleman photoelectric colorimeter. To another 10 cc. of the first dilution (the pH of which had been determined), 0.2 cc. of concentrated sulfuric acid was added, followed by hydrolysis in a boiling water bath for 15 minutes. Subsequently, the original pH was restored through addition of an appropriate amount of 30 per cent sodium hydroxide. Now one part of the hydrolyzed urine was likewise diluted with eight parts of distilled water, and 1 cc. was placed in each of the test tubes to be processed colorimetrically in the same way as the nonhydrolyzed samples. Three parallel determinations (three test tubes) were made for the nonhydrolyzed and for the hydrolyzed urines, respectively. The amounts of nonconjugated and total (nonconjugated plus conjugated) catechols, excreted in 12 hours and expressed in milligram epinephrine color equivalents (number of milligrams of epinephrine giving identical color effect), were calculated by multiplying the number of color units, read from the standard curve, with 0.008 for nonhydrolyzed and 0.016 for hydrolyzed urine.

In model tests, the chromogenicity of neither epinephrine nor norepinephrine was significantly altered by the process of hydrolysis. The supernatant fluid over the alkaline aluminum hydroxide adsorbates of nonhydrolyzed urine samples contained chromogenic material which was not present in the supernatant fluid of equally prepared adsorbates from hydrolyzed urine. Thus, it appears that hydrolysis had made previously conjugated and therefore unadsorbable catechols suitable for adsorption and colorimetry.

Neither the addition of the preservative nor allowing the specimens of urine to stand at room temperature for as long as 24 hours caused any significant alteration of the colorimetric results.

**RESULTS**

(a) **Under Normal Conditions.** One hundred and fifty-nine tests, carried out on the night urines of 69 hospitalized normotensive patients in whom no major abnormality of sympathoadrenal function was suspected, yielded values which corresponded rather closely to 29 analo-

### Table 1.—Catechol Excretion in 12 Hour Night Urine Specimens

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Normal students</td>
<td>29</td>
<td>29</td>
<td>2.1 (0.9-3.4)</td>
<td>5.0 (1.6-9.7)</td>
<td>2.4 (1.0-4.0)</td>
</tr>
<tr>
<td>Normotensive hospitalized patients</td>
<td>69</td>
<td>159</td>
<td>2.2 (0.2-11.2)</td>
<td>4.4 (0.4-19.2)</td>
<td>2.0 (1.0-7.8)</td>
</tr>
<tr>
<td>Hypertension, group I (bl. pr. 170/100 and above)</td>
<td>16</td>
<td>52</td>
<td>2.8 (0.6-15.3)</td>
<td>5.2 (1.5-17.0)</td>
<td>1.9 (1.0-4.8)</td>
</tr>
<tr>
<td>Hypertension, group II (bl. pr. 150/90 to 170/100)</td>
<td>15</td>
<td>27</td>
<td>2.8 (0.8-6.4)</td>
<td>4.5 (0.9-11.2)</td>
<td>1.6 (1.0-2.7)</td>
</tr>
<tr>
<td>Recent myocardial infarction</td>
<td>5</td>
<td>16</td>
<td>3.9 (0.9-9.6)</td>
<td>6.7 (1.5-15.6)</td>
<td>1.7 (1.0-5.0)</td>
</tr>
<tr>
<td>Congestive cardiac failure</td>
<td>12</td>
<td>32</td>
<td>2.3 (0.4-7.4)</td>
<td>3.6 (0.6-9.7)</td>
<td>1.6 (1.3-2.9)</td>
</tr>
<tr>
<td>Pregnancy (6th to 9th month)</td>
<td>9</td>
<td>13</td>
<td>2.0 (0.6-6.1)</td>
<td>3.7 (1.0-6.8)</td>
<td>1.9 (1.1-6.7)</td>
</tr>
<tr>
<td>Panhypopituitarism</td>
<td>3</td>
<td>19</td>
<td>0.7 (0.4-1.2)</td>
<td>1.0 (0.4-1.8)</td>
<td>1.4 (1.0-3.0)</td>
</tr>
</tbody>
</table>

### Table 2.—Day and Night Catechol Excretion by Hospitalized Patients

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7 a.m. to 7 p.m...........</td>
<td>10</td>
<td>34</td>
<td>2.5 (0.5-4.6)</td>
<td>5.3 (1.2-12.0)</td>
<td>2.1 (1.1-5.0)</td>
</tr>
<tr>
<td>7 p.m. to 7 a.m........</td>
<td>10</td>
<td>34</td>
<td>2.7 (0.7-6.3)</td>
<td>5.2 (0.7-13.4)</td>
<td>1.9 (1.1-5.0)</td>
</tr>
</tbody>
</table>
gious tests done on the night urines of 29 healthy students (table 1).

(b) Day and Night Values. No significant difference was found between the day and night catechol excretion in 34 tests on 10 hospitalized individuals (table 2).

(c) Effect of Emotional Tension. In 29 medical students, the urinary catechol excretion during the night preceding an examination did not differ significantly from that observed in midsemester (table 3). Neither did tests on 14 students show any difference between the excretion during four-hour periods which were filled with written examinations, and that during corresponding four-hour periods on ordinary class days (table 3); blood pressure and heart rate, on the other hand, were significantly elevated immediately before the examinations.

(d) Findings in Arterial Hypertension (table 1). Fifty-two tests were carried out on the night urines of 16 hospitalized patients with moderate to severe, but uncomplicated hypertension (blood pressure from 170/100 up) and 27 tests on the next urines of 15 patients with mild hypertension (150/90 to 170/100). There were no significant differences in catechol excretion between these two hypertensive groups, nor between the hypertensive and the normotensive groups. In cases in which tests were carried out repeatedly, the day-to-day variations proved considerable, both among normotensive and hypertensive individuals. (See fig. 1.)

(e) Effect of Thoracolumbar Sympathectomy. Twelve-hour night specimens were taken from two hypertensive women before operation, after left thoracolumbar sympathectomy, and later after additional right thoracolumbar sympathectomy (fig. 1 and table 4). In case F. R., only hydrolyzed urine samples were tested. Significant diminutions of the average catechol excretion were observed following left sympathectomy in both cases, but in the period following the final contralateral operation no further diminution of the readings was noted. In case F. R., the values even returned to and above the preoperative level. The blood
 catechol concentrations varied rather widely both before and after the operations without showing any postoperative downward trend, except temporarily in case E. B. (fig. 1).

In both patients F. R. and E. B. the removed sympathetic tissue was colorimetrically examined (table 4). The two first removed left sympathetics contained 2,740 and 3,111 color units (each unit equalling 0.001 microgram of epinephrine) per gram, respectively,
while the later removed right sympathetic tissue contained only 242 and 301 color units per gram, respectively. Thus, the last removed sympathetic tissues contained in both cases approximately 90 per cent less catechols than their earlier extirpated counterparts.

(f) Effect of Infusions of Epinephrine and Norepinephrine. On two different days, a normal volunteer received intravenous infusions of epinephrine (0.93 mg.) and norepinephrine (1.86 mg.) over periods of one and two hours, respectively. The urine was collected by catheter (table 5). During the epinephrine infusion, the catechol excretion was slightly increased; during the infusion of norepinephrine, on the other hand, it appeared to be diminished.

(g) Findings in Uremia. In 10 patients with uremia and high blood catechol concentrations (table 6), a total of 28 night urine specimens was examined. The average amount of catechols recovered from the nonhydrolyzed urines was only slightly below the normal average but that from the hydrolyzed urines was about 25 per cent below normal.

(h) Findings after Recent Myocardial Infarction. In five cases of myocardial infarction, 16 twelve-hour night urine specimens were collected on the days on which the infarctions occurred and/or during the following few days (table 1). The readings were high in one case but near normal in the others.

(i) Findings in Congestive Heart Failure. In 12 patients with congestive heart failure (32 tests) the nonhydrolyzed urines gave a normal average reading; the average reading of the hydrolyzed specimens was slightly below normal (table 1).

(j) Influence of Pregnancy. In nine women in the sixth to ninth month of pregnancy, the values were not significantly different from those found in nonpregnant women (table 1).

(k) Influence of Hypopituitarism. In nine tests on three patients, the catechol excretion was consistently subnormal (table 1).

**DISCUSSION**

In accordance with the findings of Nuzum and Bischoff, the statement of Kroneberg and Schümann that the ratio of hydrolyzed to nonhydrolyzed catechols is increased in the urine of hypertensive individuals could not be confirmed. We did not find any significant difference between the urines of normotensive persons (hospitalized and nonhospitalized) on the one hand, and those of mildly or severely hypertensive patients on the other, except

*Color units (each = 0.001 microgram epinephrine) per cc.
† Nonprotein nitrogen.

**TABLE 6.—Total 12-Hour Night Urine- and Blood Catechols in Renal Uremic Patients**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>N.P.N. mg%</th>
<th>No. Urine Specimens</th>
<th>12-Hour Night Urine Catechols</th>
<th>Blood Catechols c.u./cc.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonhydrolyzed Urine (Avg. and Range)</td>
<td>Hydrolyzed Urine (Avg. and Range)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Mg. Epinephrine Color Equivalents)</td>
<td>(Mg. Epinephrine Color Equivalents)</td>
</tr>
<tr>
<td>1</td>
<td>178</td>
<td>4</td>
<td>2.0 (1.1-4.3)</td>
<td>2.5 (1.1-5.3)</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>4</td>
<td>1.8 (0.9-2.6)</td>
<td>4.3 (2.0-6.8)</td>
</tr>
<tr>
<td>3</td>
<td>?</td>
<td>3</td>
<td>2.0 (1.0-4.0)</td>
<td>4.9 (1.8-7.9)</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>1</td>
<td>2.0 (2.0-2.0)</td>
<td>2.0 (2.0-2.0)</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>2</td>
<td>0.7 (0.5-0.9)</td>
<td>0.8 (0.8-0.8)</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>1</td>
<td>0.8 (0.8-0.8)</td>
<td>0.8 (0.8-0.8)</td>
</tr>
<tr>
<td>7</td>
<td>?</td>
<td>3</td>
<td>0.7 (0.0-1.8)</td>
<td>1.1 (0.0-2.8)</td>
</tr>
<tr>
<td>8</td>
<td>86</td>
<td>6</td>
<td>1.8 (1.1-3.4)</td>
<td>3.4 (1.6-7.8)</td>
</tr>
<tr>
<td>9</td>
<td>120</td>
<td>2</td>
<td>3.8 (2.5-5.1)</td>
<td>6.1 (4.5-7.8)</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>2</td>
<td>2.1 (0.8-3.4)</td>
<td>3.1 (0.9-5.3)</td>
</tr>
<tr>
<td>Total....</td>
<td>28</td>
<td></td>
<td>1.9 (0.0-5.1)</td>
<td>3.2 (0.0-7.9)</td>
</tr>
<tr>
<td>Normal....</td>
<td>159</td>
<td></td>
<td>2.2 (0.2-11.2)</td>
<td>4.4 (0.4-19.2)</td>
</tr>
</tbody>
</table>
perhaps a slight augmentation of the non-hydrolyzed catechols in the latter groups.

We do not believe that our essentially negative findings disprove the possibility of an increased sympathetic secretion of norepinephrine into the cardiovascular muscular tissue in arterial hypertension and in states of emotional excitement. A substantial portion of the locally discharged catecholamines is probably destroyed right on the spot and the amounts of active sympathomimetic catecholamines which can be assumed to be responsible for the clinical cardiovascular tone at a given time form only a small fraction of the total catechols excreted in the urine. Their fluctuations may be easily overshadowed by the bulk of pharmacodynamically inactive chromogenic compounds. Norepinephrine, because of its relatively weak color intensity, is particularly unlikely to markedly influence the total color readings.

Only about 10 per cent of infused epinephrine were colorimetrically recovered in the urine during the infusion while infused norepinephrine could not be demonstrated at all in the specimens collected during and within four hours after the infusion. These results are not surprising since it was found by Bacq\textsuperscript{11} that infused epinephrine is rapidly stored by the tissues and then again slowly but only partially released so that free active epinephrine appears in the urine only in small quantities within an hour after infusion. Conjugated inactive epinephrine is excreted even more slowly. Not more than 5 per cent were recovered over a period of eight hours. The excess output of active norepinephrine during intravenous infusion of this catecholamine, as observed by von Euler and Luft,\textsuperscript{13} was only 1.5 to 2.5 per cent of the infused quantity. Thus, the time element, as well as enzymatic destruction of part of the sympathomimetic catechols in the body, constitute additional factors of uncertainty in attempts to evaluate adrenosympathetic neurosecretory activity by the determination of total urinary catechol excretion.

The markedly reduced catechol concentration in the sympathetic nerve tissue, excised two weeks after contralateral sympathectomy, suggests the possibility that after removal of major sections of sympathetic nervous tissue, the remaining portions of the sympathetic nervous system intensify their catechol-discharging activity at the expense of their reserves, thus maintaining a more or less undiminished total catechol output, even though from differently distributed areas, possibly including the brain.\textsuperscript{15} The relatively uncharacteristic behavior of the blood and urine catechols would seem to be consistent with this hypothesis.

Our observations on patients with recent myocardial infarction and with congestive heart failure do not suggest any specific abnormalities of catechol excretion. The same can be said of pregnancy. The markedly subnormal catechol excretion in hypotuitarism, a condition which is usually accompanied by postural hypotension, may constitute an analogy to the low excretion of norepinephrine and epinephrine which was observed by Luft and von Euler\textsuperscript{14} in patients with orthostatic hypotension.

In 10 renal uremic patients, the colorimetric urine readings suggested an impairment of excretion, especially of the conjugated (hydrolyzed) catechols. This is of interest insofar as it seems to explain the regularly observed elevation of the blood catechol concentration in uremia\textsuperscript{15} and the resulting "false positive" benzodioxane and Regitine tests in such patients.\textsuperscript{14, 17, 18}

**Summary**

In 530 urine specimens, obtained from 169 subjects (usually for the period of 7 p.m. to 7 a.m.), the total catechol excretion was determined colorimetrically by the method of Shaw. Since part of the catecholamines, secreted by the adrenal medulla and by the sympathetic nerves, is probably destroyed in the body, and since the urine readings include large portions of pharmacodynamically relatively inactive catechol compounds, the colorimetric results do not present a clear quantitative picture of the neurosecretory discharges of pharmacodynamically active norepinephrine and epinephrine at a given time. Part of the latter substances is excreted in a sulfonjugated, inactive, but colorimetrically re-
coverable, form. A differentiation of epinephrine and norepinephrine by means of the Shaw method is not possible, contrary to statements which have appeared in the literature.

No regularly or grossly abnormal total catechol excretions were observed in patients with arterial hypertension, congestive heart failure or myocardial infarction, or in persons in a state of emotional tension. There was no significant difference between day and night excretion. In three patients with hypopituitarism, the catechol excretion was markedly subnormal.

Infusion of epinephrine increased the total catechol readings only slightly; infused norepinephrine could not be detected in the urine by colorimetry.

Unilateral thoracolumbar sympathectomy was followed by a transient diminution of the urinary catechols, but this was not further accentuated by subsequent contralateral sympathectomy. The catechols of the sympathetic tissue, obtained at the second operation, and compared with the first excised tissue, appeared to be reduced by 90 per cent in two patients examined. This suggests that after partial sympathectomy there is a compensatory overdischarge from the remaining sympathetic elements.

In uremic patients, the excretion of the conjugated catechols was significantly diminished. This is consistent with the regular and characteristic finding of elevated, presumably retained, blood catechols in renal uremia.

**Sumario Español**

En 530 especímenes de orina obtenidos en 109 sujetos (usualmente en el período de 7 p.m. a 7 a.m.), la excreción total de catecol fue determinada calorimetricamente por el método de Shaw. Como parte de las catecolaminas, secretadas por la médula adrenal y por nervios simpáticos, es probablemente destruida en el cuerpo y como las lecturas en las orinas incluyen grandes porciones de relativamente inertes farmaco-dinámicamente compuestos de catecol, los resultados calorimétricos no representan un cuadro cuantitativo claro de las descargas neurosecretorías de norepinefrina o epinefrina farmaco-dinámicamente activa en un tiempo dado. Parte de las substancias últimas son excretadas en formas sulfococonjugasadas, inactivas, pero calorimetricamente recobrables. Una diferenciación entre epinefrina y norepinefrina por medio del método de Shaw no es posible, contrario a manifestaciones que han aparecido en la literatura.

Las excrecciones de catecol total observadas en pacientes con hipertensión arterial, decom- pensación cardíaca o infartos del miocardio, o en personas en estados de tensión emocional, no fueron regularmente o crasamente anormales. No hubo diferencia significativa entre la excreción diurna o nocturna. En tres pacientes con hipopituitarismo, la excreción de catecol fue marcadamente subnormal.

La infusión de epinefrina aumentó las lecturas de catecol total por muy poco solamente, infusión de norepinefrina no se pudo descubrir en la orina por colorimetría. Simpatectomía unilateral toracolumbar fué seguida por una diminución transitoria de catecoles urinarios pero esto no fué aumentado más con una simpatectomía contralateral. Los catecoles del tejido simpático, obtenidos en la segunda operación y comparados con los tejidos en la primera, aparecieron reducidos 90 por ciento en dos pacientes examinados. Esto sugiere que luego de una simpatectomía parcial hay una sobre-descarga compensatoria del tejido simpático restante. En pacientes urérmicos, la excreción de los catecoles conjugados fué significativamente disminuida. Esto es consistente con el hallazgo regular y característico de catecoles elevados presumiblemente retenidos en la uremia renal.

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