Measurement of Effective Renal Plasma Flow: A Comparison of Methods

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We have compared two in vitro methods and three variations of kidney background (BG) subtraction within a gamma camera method (41 examinations, 31 patients) for determination of effective renal plasma flow (ERPF) using $^{131}$I orthiodohippurate (OIH). Method I: plasma samples at 20 and 45 min after OIH injection, ERPF = dose x slope/intercept; Method II: 45-min plasma sample, ERPF = $-51.1 + 8.21x + 0.019x^2$, $x$ = dose/45-min plasma activity/l. Individual kidney and total ERPF were determined from gamma camera (GC) methods using renal uptake 1–2 min after injection. All methods were compared against Method I (previously validated against paraaminohippurate (PAH) clearances). Method II, which requires one blood sample is more accurate than GC methods. GC methods are insensitive to operator variability in placement of renal and BG regions of interest. They may be useful to follow changes in relative or total ERPF, but accurate depth correction of renal data is suggested. In vitro, blood sample-based methods are more accurate.


Many investigators have suggested modifications of the methods for measurement of effective renal plasma (ERPF) with iodine-131 ($^{131}$I) orthiodohippurate (OIH) during the past 25 yr (6). Procedures requiring four to six or more blood samples generally are not considered practical for clinical use although these methods are probably the most accurate. Among the more practical clinical methods, requiring two blood samples or fewer, there are few data available comparing the methods with each other in specific clinical situations, and the reproducibility of the methods has not been addressed adequately in the literature.

The reluctance of nephrologists and clinicians to accept the radionuclide procedures for the measurements of renal function has many causes. (6) Contributing to these, undoubtedly, is confusion concerning which method to use in a specific clinical setting. We have attempted to address this problem by comparing several of the more frequently utilized techniques of measuring effective renal plasma flow using $^{131}$I-labeled orthiodohippurate.

MATERIALS AND METHODS

Patient Selection

Patients were selected from among those with known or suspected asymmetric renal size or function of any etiology, as determined by a previous examination (sonogram, intravenous urogram, or qualitative scintigram) including obstructive uropathy and suspected renovascular disease. Thirty-four patients were studied with 45 examinations. Three patients (four examinations) were excluded from analysis because of technical failures. Two examinations in one patient were excluded because of unreasonably wide discrepancies between various measures of renal function; this patient had a serum creatinine on both occasions of 0.7, a BUN of 11 and 12 mg/dl, an ERPF by both in vitro methods near 1,000 ml/min and excellent renal function visually on the gamma camera analog images. The computer calculations of the gamma camera images, however, yielded total ERPF in the range of 300 ml/min. This patient was not very obese, but the correction factor for kidney depth clearly did not apply properly. During the analysis of two other patient samples, the high voltage on the well counter was functioning improperly. After these exclusions, we evaluated 41 examinations successfully completed in 31 patients.

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Methods Compared for Measuring ERPF

In Vitro. Method I. The clearance of orthioiodohippurate (OIH) may be estimated by obtaining two blood samples, the first at 20 min after injection of the radiotracer; (the second sample in this study was obtained at 45 min). In this simplified one-compartment model described by Blaufuss and Merrill (2), the clearance of OIH = dose x slope/intercept. This has been shown to yield acceptable values for ERPF, generally overestimating true ERPF by 10–15%. The correlation with ERPF from PAH clearances is r = 0.90, p < 0.01.

Method II. A single blood sample may be related to the ERPF as well. The concentration of OIH in the plasma at a given time after injection is inversely related to the ERPF. Tauxe et al. (3,7) explored the relationship between the reciprocal of this concentration and ERPF at various times after injection of OIH. Correlation of the observed data points with ERPF may be approximated by either quadratic or exponential curves with fitted parameters. We have chosen to examine the quadratic fit, ERPF = A + Bx + Cx^2 with coefficients A, B, and C as shown in Table 1. These coefficients are dependent on the time chosen for obtaining the plasma sample. It can be seen from Table 1 that the lowest s.e.e. is obtained for blood sampled at 44 or 45 min after injection of OIH. In this study blood was drawn at 45 min, and the coefficients A, B, and C appropriate for that sampling time were used.

In vivo. The method first described by Schlegel and Hamway (4) (and later modified by Gates et al. (8) for glomerular filtration rate measurements) was used as the reference technique. This method utilized gamma camera imaging of the kidneys after OIH injection, with the camera interfaced to a computer. The 1-2 min background and depth corrected renal uptake may be used to describe individual as well as total ERPF when compared with the injected dose. The dose is calibrated by imaging and measurement on the gamma camera and computer. The original description of the method identified a region between the lower poles of the kidneys for background correction of kidney activity.

Three variations of this in vivo method were compared by varying the regions chosen for background subtraction. The regions chosen for comparison were (Fig. 1): Method III: Between the lower poles (4); Method IV: Between the upper poles (5); and Method V: Crescent-shaped regions adjacent and lateral to each kidney (5). The measured radioactivity within each of these regions was subtracted from the activity within the renal area after normalization of counts (i.e., after multiplication by a factor to correct for the difference in the number of picture elements between the background and kidneys).

By this method (4):

\[
\text{ERPF per kidney} = \frac{\text{net kidney cpm (min 1-2)} \times 100 \times F}{\text{Dose (cpm)}},
\]

where \(F = \text{depth related correction factor} = y^2\)

and \(y = \text{"depth" of kidney} = \begin{cases} 13.3x + 0.7 \text{ cm} & \text{(right kidney)} \\ 13.2x + 0.7 \text{ cm} & \text{(left kidney)} \end{cases}\)

and \(x = \text{weight (kg)/height (cm)}\).

While individual kidney ERPF is determined by these gamma camera methods, we compared total ERPF only, in the context of this investigation, except as noted below.

Depth Correction Evaluation

The depth related correction factor "F", defined above, was employed by Schlegel et al. (4). This formula did not appear to allow for much difference in attenuation between the kidneys. We compared the percentage of renal function subtracted by the left kidney using the above formula with the percent calculated without depth correction.

Protocol

Comparison of the five described techniques in a single patient study required the following protocol:

1. 300 μCi of [131I]OIH are drawn into a 10-ml syringe with normal saline added to bring the total volume of 5.0 ml.

2. Thorough mixing is accompanied by inverting the bubble.

3. A 10-μl aliquot from the 5-ml dose, in duplicate, is set aside and measured in the well counter as the standard for the in vitro methods.

4. The dose is calibrated for the in vivo method by measuring the syringe directly on the collimator face of a predefined portion of the gamma camera, interfaced to a computer.

5. The patient is positioned prone under the camera and injected with the 5-ml dose.

6. Computer images are obtained every 15 sec for 30 min.

7. Finally, 5-ml blood samples are drawn into heparinized syringes at 20 and 45 min after the OIH injection.

After centrifugation, 1 ml of plasma from each of the blood samples is obtained in duplicate and then measured in the well counter. Both samples are used to measure ERPF by the single-compartment model of plasma disappearance of Blaufuss and Merrill (2). The 45 min sample alone is used to measure the ERPF by the method of Tauxe et al. (3,7) with coefficients appropriate for the 45-min sampling time from Table 1. The equation, therefore, is \(\text{ERPF} = -51.1 + 8.21x - 0.019x^2\) where \(x = \text{dose/(plasma CPM/liter at 45 min)}\). The 1-2 min renal uptake is measured using each of the three in vivo gamma camera method variations.

Patients for the first 29 studies were imaged for 30 min and then allowed to sit up for 15 min before the 45-min blood sample was obtained. Since differences in ERPF due to postural changes may influence the results, patients in the next 12 studies remained prone after imaging and for the 45-min

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Table 1

<table>
<thead>
<tr>
<th>Sampling time (min)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Sy \times x (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>-63.0</td>
<td>9.32</td>
<td>-0.024</td>
<td>32.81</td>
</tr>
<tr>
<td>42</td>
<td>-59.9</td>
<td>9.02</td>
<td>-0.022</td>
<td>32.66</td>
</tr>
<tr>
<td>43</td>
<td>-56.9</td>
<td>8.74</td>
<td>-0.021</td>
<td>32.55</td>
</tr>
<tr>
<td>44</td>
<td>-54.0</td>
<td>8.47</td>
<td>-0.020</td>
<td>32.48</td>
</tr>
<tr>
<td>45</td>
<td>-51.1</td>
<td>8.21</td>
<td>-0.019</td>
<td>32.44</td>
</tr>
<tr>
<td>46</td>
<td>-48.3</td>
<td>7.96</td>
<td>-0.018</td>
<td>32.45</td>
</tr>
<tr>
<td>47</td>
<td>-45.6</td>
<td>7.72</td>
<td>-0.016</td>
<td>32.50</td>
</tr>
<tr>
<td>48</td>
<td>-43.0</td>
<td>7.49</td>
<td>-0.016</td>
<td>32.58</td>
</tr>
<tr>
<td>49</td>
<td>-40.3</td>
<td>7.27</td>
<td>-0.015</td>
<td>32.71</td>
</tr>
<tr>
<td>50</td>
<td>-37.8</td>
<td>7.05</td>
<td>-0.014</td>
<td>32.87</td>
</tr>
</tbody>
</table>
and $x(1) = \text{ERPF}$ by 2 sample method. $m_a = \text{slope}$ and $b_a = \text{intercept}$ of the regression.

$r$ Values and s.e.e. were calculated for each regression. Regressions also were determined predicting Method I from a group of all four other methods. The beta-weights for predicting Method I from all the other methods were compared by the Student’s t-test.

Depth correction formulae were evaluated by paired t-tests applied to uncorrected versus depth corrected values for relative renal function.

**Precision of Processing Routines**

The variability of ERPF determination according to manual selection of renal and background regions-of-interest was tested in 14 of the examinations. One of us (MSA) selected all kidney and background regions-of-interest (ROIs) (Fig. 1). These ROI selections were repeated independently, by another one of us (EJF), to examine interobserver reproducibility. Intraobserver reproducibility for the second investigator was examined by another set of independently chosen ROI selections. Intraclass correlation coefficients ($r$) were calculated to compare intra and interobserver variability.

**RESULTS**

**Accuracy of Methods**

There is excellent correlation of the in vitro methods. Figure 2 compares the in vitro single sample method of Tauxe et al. (3) (Method II) with the two-sample method of Blaufox and Merrill (2) (Method I) as the reference standard in 41 examinations, demonstrating a slope of 0.85, an intercept of $-7$, and an $r$ value of $0.977$.
0.98. The s.e.e. is 42 ml/min. The mean ERPF value by the reference method (I) is 384 ± 222 ml/min (mean ± s.d.). By Method II, the mean ERPF is 319 ± 193 ml/min.

The correlations are not as good for any of the in vivo gamma camera variations. Figure 3 demonstrates the regression of gamma camera Method III (with the background (BG) chosen between the upper poles) against in vitro Method I. The slope is 0.84, the intercept is -62, and the r value is 0.91. The s.e.e. is 88 ml/min. The mean ERPF calculated by Method III is 261 ± 207 ml/min.

For the BG chosen between the lower poles (Method V), the slope is 0.89 with an intercept of 50 and an r value of 0.90 (Fig. 4). The s.e.e. for Method IV versus Method I is 99 ml/min. The mean ERPF calculated by Method IV is 392 ± 220 ml/min.

For Method V (BG as crescents lateral and adjacent to each kidney) versus Method I, the slope is 0.85, with an intercept of -9, and an r value of 0.90 (Fig. 5). The s.e.e. for this regression is 93 ml/min. The mean ERPF calculated by Method V is 316 ± 209 ml/min.

Table 2 summarizes the pertinent data. It must be considered that a change in position of the patient during the study would effect the results. A postural change from prone to sitting at 30 min in the first 29 studies yielded results consistent with the next 12 studies (in which patients remained prone through 45 min). This supports the appropriateness of pooling the results of all 41 studies. The s.e.e. was lowest and the r value of the regression highest for the one-sample in vitro method (Method II). The gamma camera methods all yielded less consistent results than Method II. The addition of Method II to an equation containing any
combination of the gamma camera methods improves the ability to predict the value of Method I (p < 0.001). (Further, the increment in $r^2$ due to the addition of Method II to any of the other methods is significant by the t-test on the beta-weights (p < 0.01).)

The results are similar if the one-sample method (Method II) is used as the reference standard instead of Method I. Using this hierarchy, the lowest SEE in comparison with Method II is Method I; the gamma camera methods have substantially higher s.e.e.

**Reproducibility of Processing Routine for Gamma Camera Methods**

Among the gamma camera methods, the reproducibility is very high. The inter- and intrainobserver reproducibility of manual ROI selection is demonstrated in Table 3. Perfect correlation within and between observers would be indicated by $r = 1.00$. The intraclass correlation coefficient for all three methods, regardless of observer, is $>0.980$. Among the gamma camera methods, therefore, manual selection of kidney and background ROIs does not affect inter- or intrainobserver reproducibility significantly.

**Gamma Camera Data with and Without Depth Correction**

The formulae employed by Schlegel et al. (9) to account for renal depth were reported originally by Tonnesen et al. (12). Summary data for the percent total renal function subtended by the left kidney is shown for Methods III, IV, and V, both with and without use of these depth correction formulae in Table 4. The formulae systematically increase the depth corrected relative function compared with the uncorrected data (p < 0.05), but in no instance is there a difference of $>1%$.

**DISCUSSION**

Among the issues to be resolved before nuclear medicine procedures become accepted for routine evaluation of renal function are the expense, moderate inconvenience and exposure to radiation as well as questions of the accuracy and reproducibility of these techniques. While the first three issues are likely to remain with us, we have attempted to begin to address the last two. In addition, we have examined a formula, based upon height and weight, popularly used for depth correcting raw kidney counts.

**Accuracy**

A full evaluation of the accuracy of measuring total effective renal plasma flow requires a "gold standard" for comparison. Measurement of the clearance of pa-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Method</th>
<th>0–30'</th>
<th>30–45'</th>
<th>Method</th>
<th>0–30'</th>
<th>30–45'</th>
<th>Method</th>
<th>0–30'</th>
<th>30–45'</th>
<th>Method</th>
<th>0–30'</th>
<th>30–45'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method II</td>
<td></td>
<td></td>
<td>Method III</td>
<td></td>
<td></td>
<td>Method IV</td>
<td></td>
<td></td>
<td>Method V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 29</td>
<td>0.974</td>
<td>42.8</td>
<td>0.918</td>
<td></td>
<td>0.903</td>
<td></td>
<td>0.990</td>
<td></td>
<td>0.892</td>
<td></td>
<td>91.8</td>
</tr>
<tr>
<td>n = 12</td>
<td>0.987</td>
<td>37.2</td>
<td>0.913</td>
<td></td>
<td>102.2</td>
<td></td>
<td>0.900</td>
<td></td>
<td>107.2</td>
<td></td>
<td>93.4</td>
</tr>
<tr>
<td>n = 41</td>
<td>0.977</td>
<td>41.5</td>
<td>0.907</td>
<td></td>
<td>87.8</td>
<td></td>
<td>0.897</td>
<td></td>
<td>98.6</td>
<td></td>
<td>93.1</td>
</tr>
</tbody>
</table>

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raaminohippurate (PAH) serves that function for the nephrologist and renal physiologist. PAH clearances were determined in only three patients in our series, so could not be used meaningfully. The standard deviation for measurement of ERPF has been estimated from repeat measurements of diodrast clearance to be ±38 ml/min. (9). Similar measurements for PAH clearance measurements are difficult to obtain from the literature but may be assumed to be similar.

Among the methods examined, both the two-sample and one-sample in vitro methods have been compared directly with PAH clearances. Published data suggest that the two-sample method has a slightly better correlation with PAH clearance, although it overestimates ERPF by 10–15%. Data from Blaufox and Merrill (2) show a correlation of r = 0.90 between the two-sample method and PAH clearances. Tauxe et al. (3,7) do not report directly on the correlation coefficients of the one-sample method with PAH clearances. The s.e.e. of the regression of the one sample method to PAH clearances also is not given, but is reported as 23.5 ml/min (7) when compared instead to ERPF calculated from a two-compartment model of [131I]OIH disappearance determined from multiple blood samples. The correlation of slope x dose/intercept (i.e., the two-sample method) to this measure of ERPF was slightly better in this study with a s.e.e. reported as 22.2 ml/min. As such, the two-sample method was chosen as our reference standard, but differences between the methods were clearly so small that either method may have been chosen. It was gratifying to find that our conclusions are, in fact, unaltered by choosing the one sample method as standard. The gamma camera method originally described by Schlegel and Hamway (4) was never compared directly with PAH clearances, but with more indirect indices of total ERPF. As such, this method was not considered appropriate as a choice for the reference standard of ERPF. In comparison with either in vitro method as standard, we find none of the variations of the gamma camera method as accurate for the measurement of ERPF.

Russell et al. (10) have compared several in vitro and in vivo techniques for measuring the glomerular filtration rate (GFR) using [99mTc]DTPA in protocols similar to our study. It is interesting that they conclude that the two sample in vitro technique is more accurate than the one sample method, and that both are substantially superior to the gamma camera methods for the determination of total GFR.

Reproducibility

Evaluation of the reproducibility of the methods is a complex issue. Repeat determinations of ERPF were performed in five patients (11 studies), but in most cases it was for an event reflecting a change in clinical status. As such, an evaluation of reproducibility of repeat measurements was not made in this study.

The in vivo, gamma camera methods require delineation of renal ROIs from computer-derived images. We postulated that manually derived ROIs may be a source of variability and error in determination of ERPF, but were surprised that this was not so. Presumably, if a renal ROI is large enough to include all functioning renal parenchyma, with [131I]OIH, function will be measured reproducibly. This may not be true for agents that are cleared more slowly, as for technetium-99m diethylenetriaminepentaacetic acid (11).

Depth Correction

We found no instance in which the depth correction algorithm used by Schlegel (4) [quoted from Tonnesen et al. (12)] changed the calculated relative renal function by >1%. One can show (see Appendix) that the percent change in ERPF/ERPFa due to depth corrections by Schlegel's formulae is 100x [1 – (13.2x + 0.7)^2]/

### TABLE 3
Inter and Intraobserver Reproducibility of ERPF Values

<table>
<thead>
<tr>
<th>Observer</th>
<th>Method III</th>
<th>Method IV</th>
<th>Method V</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>X</strong></td>
<td>MSA</td>
<td>EJF&lt;sub&gt;a&lt;/sub&gt;</td>
<td>EJF&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>± s.d.</td>
<td>280.6</td>
<td>285.1</td>
<td>275.8</td>
</tr>
<tr>
<td><strong>r</strong></td>
<td>0.981</td>
<td>0.986</td>
<td>0.992</td>
</tr>
</tbody>
</table>

*<sup>1</sup> X = Mean of all ERPF determination using gamma camera data and one observer (MSA or EJF<sub>a</sub> or EJF<sub>b</sub>).  
*<sup>1</sup> r = Intraclass correlation coefficient (relationship of all values for a single method regardless of observer.

### TABLE 4
Percent of Total ERPF Subtended by the Left Kidney (ERPF<sub>L</sub>) With (D) and Without (ND) the Depth Correction Algorithm for the Three Gamma Camera Methods (n = 41)

<table>
<thead>
<tr>
<th>Method III</th>
<th>Method IV</th>
<th>Method V</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>D</td>
<td>ND</td>
</tr>
<tr>
<td>Mean (%)</td>
<td>44.3</td>
<td>44.5</td>
</tr>
<tr>
<td>s.d.</td>
<td>29.1</td>
<td>29.2</td>
</tr>
</tbody>
</table>
(13.3x + 0.7)^2}. If we examine this relation for extreme values of x, we find that the maximum percent change due to the given depth correction formulae is 1.4% (see Appendix). This is well within the error of measurement and has little meaning, clinically. Gruenwald et al. (13) derived an alternative set of formulae for renal depth based on patient height and weight, but concluded they were too inaccurate for clinical use. The importance of accurate depth correction in determining relative renal function from technetium-99m DMSA studies also was emphasized by Choi et al. (14)

Proper depth correction is essential for determination of both absolute and relative renal function, even with \[^{131}\text{I}]\text{OIH. The attenuation coefficient of 364 keV photons from }^{131}\text{I} \text{in water density media (similar to soft tissue) is } \approx 0.108 \text{ per cm. For each cm of depth difference between the kidneys, therefore, } \approx 10\% \text{ error in relative and absolute renal function will be made if depth correction is not employed. In the report by Tønnessen et al. (12), the difference in kidney depths, as measured by B-mode ultrasound, is } >1 \text{ cm in } 18\% \text{ of patients. In a recent report on 150 patients examined with real time ultrasound, by Gruenwald et al. (13), this figure reached 34\%, and a figure of 33\% was reported in a series of 52 patients by Nimmon et al. (15), using A-mode ultrasound.}

CONCLUSION

1. The one-sample, in vitro method of determining ERPF with \[^{131}\text{I}]\text{OIH is more accurate than the gamma camera in vivo methods tested, when compared with a two-blood sample in vitro method as standard.}

2. While less accurate, the reproducibility of the gamma camera methods is not influenced significantly by manual selection of computer regions of interest.

3. For quantitation of relative ERPF, use of depth correction formulae determined from height and weight nomograms is inaccurate. For individual patients where changes in relative function are being followed with time, the temporal change is more important than the accuracy of the value of relative function. In such patients depth correction is unimportant. In patients in whom the value of relative or absolute function matters, the correction by height-weight formulae is inaccurate. For accurate quantitation of total or relative ERPF using the gamma camera, a more reliable method, such as an ultrasound or lateral scintigrams should be used.

For clinical measurement of total ERPF using radionuclides, a single blood sample obtained at ~45 min after injection of \[^{131}\text{I}]\text{OIH combines the optimum virtues of accuracy and practicality among the methods tested for measurement of total ERPF. We do not recommend gamma camera methods alone for measurement of total ERPF.}

\[ y_L = 13.2x + 0.7 \]
\[ y_R = 13.3x + 0.7 \]
\[
\Delta \text{ERPFL} = 100 \times \left(1 - \frac{(13 - 2x + 0.7)^2}{(13.3x + 0.7)^2}\right) \tag{1}
\]

For two extreme cases:

(a) Very obese: Weight 300 lb = 136.2 kg  
\[ x = \frac{\text{weight}}{\text{height}} = \frac{136.2}{154.4} = 0.8937 \]
\[ = 136.2 \text{ kg; 154.2 cm} \]

(b) Very thin: Weight 154 lbs = 70 kg  
\[ x = 0.3533 \]
\[ = 70 \text{ kg; r198.1 cm} \]

Substituting in (1)

For very obese case:  
\[
\frac{\Delta \text{ ERPFL}}{\text{ERPFL}} = 1.4\%
\]

For very thin case:  
\[
\frac{\Delta \text{ ERPFL}}{\text{ERPFL}} = 1.3\%
\]

By the depth correction nomogram of Schlegel (see text):

\[
\text{ERPFL} = \frac{100 \times (\text{CPM}_{L} 1-2 \text{ min}) \times y_{L}}{\text{dose}}
\]
\[
\text{ERPFR} = \frac{100 \times (\text{CPM}_{R} 1-2 \text{ min}) \times y_{R}}{\text{dose}}
\]
\[
\frac{\text{ERPFL}}{\text{ERPFR}} = \frac{\text{CPM}_{L} y_{L}}{\text{CPM}_{R} y_{R}}
\]

Whereas \[
\frac{\text{ERPFL}}{\text{ERPFR}} \text{(uncorrected)} = \frac{\text{CPM}_{L}}{\text{CPM}_{R}}
\]
\[
\Delta \text{ change in } \frac{\text{ERPFL}}{\text{ERPFR}} \text{ due to correction algorithm:}
\]
\[
= 100 \times \left(1 - \frac{y_{L}}{y_{R}}\right)
\]

REFERENCES

3. Tauxe WH, Dubovsky EV, Kidd T, Jr., et al. New formulas for the calculation of effective renal plasma


