A simple method for determination of antipyrine clearance

Antipyrine clearance (Cl\textsubscript{ap}) is widely used for assessment of microsomal liver function. The usual procedure involves collection of 4 to 7 samples of plasma or saliva obtained during 24 to 48 hr. To determine whether this procedure could be simplified, it was compared with one based on a single sample (sCl\textsubscript{ap}) and an estimated volume of distribution (V\textsubscript{d}). V\textsubscript{d} was estimated from body weight in kilograms (BW), height in centimeters (BH), age in years, and sex, or, in some cases, to be 40 l. The agreement between the values of Cl\textsubscript{ap} and sCl\textsubscript{ap} increased with the time of the single sample and the two clearance estimates were nearly identical in all cases when the sample was taken after 18 hr. The method used for assessment of V\textsubscript{d} had only a small influence on the agreement. It is suggested that antipyrine clearance (in ml/min) is estimated as

\[
x\text{Cl}_{ap} = \frac{\ln(D/V_d)}{t} \times c_i, \text{ where } D \text{ is the dose of antipyrine (in mg), } c_i \text{ the concentration of antipyrine (in mg/l) at sampling time } t (\text{in min}), t \text{ should be about 1440 min (24 hr), and } V_d \text{ (in l) is calculated } = 0.3625 \times BW + 0.0272 \times BH - 0.1387 \times age - 0.0272 (\text{women}) \text{ or } 0.3625 \times BW + 0.2239 \times BH - 0.1387 \times age - 14.47 (\text{men}). \text{ Little information is lost, however, if a fixed volume of 40 l is used. Then, if the dose is 1 g, } c_i \text{ is expressed in milligrams per liter, and the sampling time is 24 hr, } x\text{Cl}_{ap} = (3.28 - \ln c_i) \times 28 \text{ ml/min.}
\]

Martin Dissing, M.D., Henrik Enghusen Poulsen, M.D., Per Buch Andreasen, Ph.D., and Niels Tygstrup, Ph.D. Copenhagen, Denmark

Medical Department A, Division of Hepatology, Rigshospitalet

Few attempts have been made to define the optimal number and times of sampling blood or saliva for estimation of hepatic elimination rate of model substances in clinical pharmacology and hepatology. The conventional 45-min sampling time used in the Bromsulphalein retention test is mainly empirically founded.\textsuperscript{10} Galactose elimination capacity has been measured as 45 and 60 min after galactose administration.\textsuperscript{31} It was later shown that reducing the number of blood samples for estimation of galactose elimination capacity leads to large deviations from measurements based on the standard method.\textsuperscript{6} A different approach has been used with investigations of single-injection technique of indocyanine green. The indocyanine green concentration in blood 6 min after dosing discriminated better between healthy subjects and subjects with liver disorders than did a method using the half-life (1/2) of indocyanine green and based on multiple samples.\textsuperscript{12} The aminopyrine breath test was introduced as a single-sample test,\textsuperscript{9} but this approach has been questioned by some, who found results based on multiple breath samples more informative.\textsuperscript{3-4}
### Table I. Results of linear regression analyses of Cl_{AP} based on multiple samples and sCl_{AP}.

<table>
<thead>
<tr>
<th>Method of V_{c} prediction*</th>
<th>Sampling interval after drug administration</th>
<th>No of samples</th>
<th>Correlation coefficient</th>
<th>Slope of regression line</th>
<th>Intercept of regression line</th>
<th>Residual variance (s²)</th>
<th>Mean (SD) Cl_{AP} (m/min)</th>
<th>Mean (SD) sCl_{AP} (m/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruce</td>
<td></td>
<td>0.4-5</td>
<td>135</td>
<td>0.80</td>
<td>1.14</td>
<td>4.69</td>
<td>282</td>
<td>39.3 (28.0)</td>
</tr>
<tr>
<td>Hume</td>
<td></td>
<td>0.78</td>
<td>1.14</td>
<td>7.74</td>
<td>317</td>
<td>30.0 (19.0)</td>
<td>41.9 (28.5)</td>
<td></td>
</tr>
<tr>
<td>Own formula</td>
<td></td>
<td>0.83</td>
<td>1.27</td>
<td>-10.2</td>
<td>293</td>
<td>28.2 (19.6)</td>
<td>29.4 (44.7)</td>
<td></td>
</tr>
<tr>
<td>40 l</td>
<td></td>
<td>0.60</td>
<td>1.36</td>
<td>-11.5</td>
<td>1279</td>
<td>35.4 (20.3)</td>
<td>36.6 (20.9)</td>
<td></td>
</tr>
<tr>
<td>Bruce</td>
<td></td>
<td>0.92</td>
<td>0.98</td>
<td>5.19</td>
<td>70.0</td>
<td>30.8 (19.5)</td>
<td>30.5 (22.1)</td>
<td></td>
</tr>
<tr>
<td>Hume</td>
<td></td>
<td>0.91</td>
<td>0.98</td>
<td>6.45</td>
<td>75.7</td>
<td>30.1 (16.3)</td>
<td>33.1 (16.1)</td>
<td></td>
</tr>
<tr>
<td>Own formula</td>
<td></td>
<td>0.93</td>
<td>1.05</td>
<td>-1.90</td>
<td>70.2</td>
<td>31.4 (16.3)</td>
<td>33.6 (16.0)</td>
<td></td>
</tr>
<tr>
<td>40 l</td>
<td></td>
<td>0.78</td>
<td>1.16</td>
<td>-5.03</td>
<td>329</td>
<td>31.0 (17.3)</td>
<td>33.6 (17.7)</td>
<td></td>
</tr>
<tr>
<td>Bruce</td>
<td></td>
<td>0.97</td>
<td>0.94</td>
<td>3.39</td>
<td>76.7</td>
<td>28.6 (16.3)</td>
<td>27.6 (15.1)</td>
<td></td>
</tr>
<tr>
<td>Hume</td>
<td></td>
<td>0.97</td>
<td>0.95</td>
<td>3.65</td>
<td>15.3</td>
<td>27.5 (15.2)</td>
<td>27.5 (15.2)</td>
<td></td>
</tr>
<tr>
<td>Own formula</td>
<td></td>
<td>0.97</td>
<td>1.03</td>
<td>-0.84</td>
<td>15.5</td>
<td>28.1 (15.3)</td>
<td>27.5 (15.3)</td>
<td></td>
</tr>
<tr>
<td>40 l</td>
<td></td>
<td>0.92</td>
<td>1.10</td>
<td>3.01</td>
<td>61.7</td>
<td>28.6 (16.3)</td>
<td>27.5 (15.2)</td>
<td></td>
</tr>
<tr>
<td>Bruce</td>
<td></td>
<td>0.98</td>
<td>0.91</td>
<td>1.37</td>
<td>8.0</td>
<td>28.6 (16.3)</td>
<td>27.5 (15.3)</td>
<td></td>
</tr>
<tr>
<td>Hume</td>
<td></td>
<td>0.98</td>
<td>0.91</td>
<td>1.66</td>
<td>7.6</td>
<td>28.6 (16.3)</td>
<td>27.5 (15.3)</td>
<td></td>
</tr>
<tr>
<td>Own formula</td>
<td></td>
<td>0.98</td>
<td>0.98</td>
<td>-0.63</td>
<td>8.7</td>
<td>25.8 (15.3)</td>
<td>25.9 (16.7)</td>
<td></td>
</tr>
<tr>
<td>40 l</td>
<td></td>
<td>0.97</td>
<td>0.98</td>
<td>1.65</td>
<td>16.7</td>
<td>25.8 (14.6)</td>
<td>25.9 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Bruce</td>
<td></td>
<td>0.99</td>
<td>0.94</td>
<td>1.34</td>
<td>4.4</td>
<td>25.8 (14.6)</td>
<td>25.9 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Hume</td>
<td></td>
<td>0.99</td>
<td>0.94</td>
<td>1.31</td>
<td>4.5</td>
<td>25.8 (14.6)</td>
<td>25.9 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Own formula</td>
<td></td>
<td>0.99</td>
<td>1.02</td>
<td>0.43</td>
<td>5.1</td>
<td>25.8 (15.3)</td>
<td>25.9 (15.3)</td>
<td></td>
</tr>
<tr>
<td>40 l</td>
<td></td>
<td>0.97</td>
<td>1.01</td>
<td>-0.26</td>
<td>14.3</td>
<td>25.8 (14.9)</td>
<td>26.1 (15.9)</td>
<td></td>
</tr>
<tr>
<td>Bruce</td>
<td></td>
<td>0.97</td>
<td>0.98</td>
<td>1.36</td>
<td>4.3</td>
<td>15.1 (9.2)</td>
<td>15.1 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Hume</td>
<td></td>
<td>0.97</td>
<td>0.90</td>
<td>1.63</td>
<td>4.9</td>
<td>15.1 (9.2)</td>
<td>15.3 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Own formula</td>
<td></td>
<td>0.98</td>
<td>0.97</td>
<td>0.59</td>
<td>4.3</td>
<td>15.1 (9.9)</td>
<td>15.2 (9.9)</td>
<td></td>
</tr>
<tr>
<td>40 l</td>
<td></td>
<td>0.97</td>
<td>0.99</td>
<td>0.99</td>
<td>6.9</td>
<td>15.1 (10.2)</td>
<td>15.1 (10.2)</td>
<td></td>
</tr>
</tbody>
</table>

*For explanation of methods of prediction, see Material and methods section of text.

Anispyrine clearance (Cl_{AP}) is widely used for quantitative assessment of microsomal liver function. In all studies of antipyrine metabolism, the clearance of the drug is estimated from the plasma or salivary decay based on four to seven samples obtained between 3 and 48 hr after 1 to 2 gm antipyrine by mouth or vein.

Our purpose was to determine whether any information is lost in the measurement of antipyrine elimination when only one sample is used for its calculation.

**Material and methods**

Cl_{AP} was estimated in 142 patients (77 women and 65 men) during the period 1978 to 1981. The men and women were of comparable age (46.4 ± 18.0 and 41.5 ± 12.6 yr, mean ± SD), but the women were shorter (165 ± 8 cm) and weighed less (61.7 ± 10.9 kg) than the men (176 ± 8 cm and 77.2 ± 15.3 kg). Antipyrine was given by mouth (n = 98) or vein (n = 44) in a dose of 15 mg/kg body weight (BW) and plasma samples of 5 ml were obtained about 3, 6, 12, 24, and 36 hr and sometimes also more than 42 hr after antipyrine. Antipyrine was measured in duplicate by spectrophotometry or gas-liquid chromatography.

Cl_{AP} was calculated as:

\[
Cl_{AP} = \frac{k \cdot V_{d}}{c_{0}}, \quad \text{where } k = \frac{dV}{dt} \text{ and } V_{d} = \frac{D}{c_{0}}
\]

where \(c\) is concentration, \(t\) is time, \(k\) is the elimination constant, estimated as the slope of the linear regression of \(\ln c\) on \(t\), \(V_{d}\) is the apparent volume of distribution, \(D\) is the dose of antipyrine given, and \(c_{0}\) is the extrapolated concentration at zero time. The simplified, one-sample clearance (sCl_{AP}) was in principle calculated in the same way, except that \(c_{0}\) was estimated from an assumed value of \(V_{d}\) and the elimination constant was assessed from \(c_{0}\) and \(c_{t}\). The equation used was:

\[
\text{sCl}_{AP} = \frac{\ln(1 + V_{d}t)}{c_{0}} \quad \text{or} \quad \text{sCl}_{AP} = V_{d} \cdot \frac{\ln t}{c_{0}}
\]
where t is time of sampling, $c_t$ the corresponding concentration, and other symbols are as above.

$C_{AP}$ and $sC_{AP}$ were calculated in all 142 patients. In each patient $sC_{AP}$ was determined for six different intervals, separated by periods 4.8, 10.8, 18, 27, and 42 hr after antipyrine dosing, whenever a sample taken within the interval was available. Samples with concentration values below 10.6 $\mu$mol/l (2 mg/l) were discarded. For intervals during which several samples had been taken, one was selected at random for the calculations. The assumed total body water (TBW) in liters (equivalent to $V_B$) was estimated in the four following different ways, where body weight (BW) is measured in kilograms, body height (BH) in centimeters, and age is given in years: (1) From the formula of Bruce et al.:  

$$
\text{TBW} = 0.40 \times \text{BW} + 0.23 \times \\
\text{BH} - 0.056 \times \text{age} + 12.1 \text{ for men}
$$

$$
\text{TBW} = 0.24 \times \text{BW} + 0.20 \times \\
\text{BH} - 0.03 \times \text{age} - 13.9 \text{ for women}
$$

(2) From the formula of Hume and Weyers:  

$$
\text{TBW} = 0.296785 \times \text{BW} + 0.192786 \times \\
\text{BH} - 14.012934 \text{ for men}
$$

$$
\text{TBW} = 0.183809 \times \text{BW} + 0.344547 \times \\
\text{BH} - 34.270121 \text{ for women}
$$

(3) From a multiple linear regression analysis of age, BH, and BW on $V_B$ of the group of women and men from the present material:  

$$
\text{TBW} = 0.3625 \times \text{BW} + 0.2239 \times \\
\text{BH} - 0.1387 \times \text{age} - 14.47 \text{ for men}
$$

$$
\text{TBW} = 0.2363 \times \text{BW} + 0.1962 \times \\
\text{BH} - 0.0272 \times \text{age} - 10.26 \text{ for women}
$$

(4) From a fixed $V_B$ of 40/ (the mean $V_B$ of the 142 patients calculated from the antipyrine elimination/time curve was 40.8 l).  

Regression analysis of $sC_{AP}$ on $C_{AP}$ was performed by the least-square method. The values of $C_{AP}$ and $sC_{AP}$, from a given set of data were examined for correlation since the concentration measurement on which the $sC_{AP}$ is based is also used together with the remaining measurements to calculate $C_{AP}$. This correlation was analyzed by calculation of the correlation coefficient from the approximate variances and the covariance of $C_{AP}$ and $sC_{AP}$ for a large number of combinations (960) covering the range of sample periods, $V_B$, clearances, coefficients of variation of $V_B$, and measured antipyrine concentrations. These correlation coefficients, due to the interdependence, ranged from 0.01 to 0.30. Accordingly, for high correlations between $C_{AP}$ and $sC_{AP}$, i.e., r values above 0.90, this interdependence introduces a bias of under 2% ($0.3^2 \times [1 - 0.9^2]$) of r$^2$. From the regression of $sC_{AP}$ on $C_{AP}$, it is possible to reveal two kinds of deviation of $sC_{AP}$ from $C_{AP}$: first, a systemic deviation of $sC_{AP}$ from $C_{AP}$, as indicated by a deviation of the intercept from zero and a deviation of the slope from one and second, a random deviation between the two estimates as expressed by the residual variance of the regression.

**Results**

The calculations resulted in one $C_{AP}$ for each of the 142 patients corresponding to four, five, or six $sC_{AP}$s for each of the four ways of estimating the $V_B$ (Table I). The plots of each set of $sC_{AP}/C_{AP}$ are shown in Fig. 1 and the results of the corresponding regression analysis are summarized in Table I.

When the single sample used for calculation of $sC_{AP}$ was obtained early, i.e., between 3 and 18 hr after antipyrine dosing, there was systematic deviation of $sC_{AP}$ from $C_{AP}$ so that $sC_{AP}$ overestimates $C_{AP}$, as indicated by a regression coefficient greater than one. When the sample was obtained more than 18 hr after drug, the regression coefficient approximates unity, indicating close agreement between both clearance calculations. Accordingly, the random variation between the two ways of estimating antipyrine clearance decreases with increasing amount of time before the sample is drawn, as indicated by the rapid decrease of the residual variance (Table I).

Antipyrine elimination was sufficiently slow in only 51 of the 142 patients (15.1 $\pm$ 9.9 ml/min) to permit the development of antipyrine concentrations above the detection limit of 10.6 $\mu$mol/l (2 mg/l) 42 hr or more after antipyrine dosing (Table I). Correlation and regression analyses of $sC_{AP}$ on $C_{AP}$ in this group revealed that the correlation coefficient
was highest and the residual variance lowest when the sample used for sCl$_{AP}$ calculation was obtained between 27 and 42 hr after dosing.

**Discussion**

Our study shows that antipyrine clearance can be estimated from one sample of plasma antipyrine, without systematic deviation from the clearance determined from multiple samples, and with a very small random variation, provided that the single sample is obtained more than 18 hr after antipyrine. Extension of the sampling interval beyond 42 hr did not improve the agreement between sCl$_{AP}$ and Cl$_{AP}$. It is therefore concluded that the sample should be obtained at about 24 hr after dosing.

Determination of antipyrine clearance by the one-sample method is simpler than that by multiple samples and almost as reliable. It should, however, be kept in mind that while the multiple-sample method reveals errors (analytical, sampling time, recording, etc.), resulting in a widely deviating point on the concentration-time plot, this is not the case with the singlesample method. The one-sample test is especially applicable in investigations in which each subject serves as his own control and where no alterations in V$_D$ are expected. On the other hand, the test cannot be used in studies in which a measurement of V$_D$ from the antipyrine data is needed.

It is well known that saliva and plasma concentrations of antipyrine correlate closely.$^{14,15}$ The one-sample test can therefore probably be
used noninvasively. In our experience the one-
sample saliva test can be carried out by patients
themselves with the help of written instructions,
so that the test can be used for screening of a
large number of patients, including outpatients.

We thank licensed technician Aage Valund for
help with the statistical analyses.

References
1. Brodie BB, Atchelus J, Soberman R, Levy BH: The estimation of antipyrine in biological mate-
2. Bruce A, Anderson M, Arvidsson B, Isakson
B: Body composition. Prediction of normal body potassium, body water and body fat in adults on
the basis of body height, body weight and age.
3. Gikatlov I, Bincher J: Dose dependence of the
14C-aminopyrine breath test. Eur J Clin Pharma-
4. Henry DA, Sharpe G, Chaplain S, Cartwright S,
Kitchingman G, Bell GD, Langman MJS: The
14C-aminopyrine breath test. A comparison of
different forms of analysis. Br J Clin Pharmacol
8:539-545, 1979.
5. Hepner GW, Vesell ES: Quantitative assessment of
hepatic function by breath analysis after oral
administration of 14C-aminopyrine. Ann Intern
6. Hoornije SJ, Gipo CH: Is simplification of the
galactose elimination (GEC) test allowed? Neth
7. Hume R, Weyers E: Relationship between total
body water and surface area in normal and obese
8. Meyer SL: Data analysis for scientists and en-
antipyrine in plasma. J Pharm Pharmacol 25:
function. VI A., The pharmacological behavior of
certain phthalein dyes. B. The value of se-
lected phthalein compounds in the estimation of
hepatic function. J Pharmacol Exp Ther 24:
11. Tygstrup N: The galactose elimination capacity
in relation to clinical and laboratory findings in
patients with cirrhosis. Acta Med Scand 175:
12. Töve J, Brügmann E, Dummler W: The analysis of
strain loading tests for the assessment of the
liver function. Acta Hepat Gastroenterol
13. Vesell ES: The antipyrine test in clinical pharma-
cology: conceptions and misconceptions. CLIN
14. Vesell ES, Passananti GT, Glenwright PA, Dvorchik BH: Studies on the disposition of an-
tipyrine, aminopyrine and phenacetin using
plasma, saliva and urine. CLIN PHARMACOL
15. Welch RM, DeAngelis RL, Wingfield M, Farmer
TW: Elimination of antipyrine from saliva as a
measure of metabolism in man. CLIN PHARMA-