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Partition Coefficients of Volatile Anesthetics
in Krebs’ Solution

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Partition coefficients of volatile anesthetics between Krebs’ solution and air were determined at 37 C. The mean values obtained were: enflurane, 0.74; diethyl ether, 11.9; fluroxene, 0.81; halothane, 0.75; isoflurane, 0.35; methoxyflurane, 3.8. (Key words: Anesthetics, volatile, enflurane; Anesthetics, volatile, ether; Anesthetics, volatile, fluroxene; Anesthetics, volatile, halothane; Anesthetics, volatile, isoflurane; Anesthetics, volatile, methoxyflurane; Solubility, partition coefficients.)

During a study of the action of anesthetics at the neuromuscular junction of isolated muscles bathed in Krebs’ solution, gas—liquid partition coefficients were needed. A survey of the literature yielded values for water, blood, or 0.9 percent sodium chloride but not for Krebs’ solution itself. Therefore we undertook to measure the partition coefficients for selected anesthetics between air and Krebs’ solution.

Methods

There are three parts to the assay: measurements of concentrations of the anesthetics in the liquid phase and in the gaseous phase, and finally comparison of values obtained from equilibrated gas—liquid systems. We discuss these in turn.

Krebs’ Solution Analysis

Liquid samples were analyzed as described by Rutledge et al.2 Specifically, a 5-ml sample from each flask was pipetted below 2 ml of heptane (or pentane when methoxyflurane was the anesthetic being examined) in a 10-ml centrifuge tube. The centrifuge tube was shaken for 10 minutes and centrifuged for 5 minutes. A 30-µl sample of the heptane layer was drawn into a 50-µl syringe and then injected into a gas chromatograph for analysis. To establish calibration curves, selected amounts of each anesthetic to be analyzed were injected by a 100-µl gas-tight syringe into flasks of measured volume (of approximately 150 ml), filled with Krebs’ solution,† and containing ten glass mixing beads. A screw cap was devised to allow the input of anesthetic with overflow of an equal volume of Krebs’ solution while avoiding the introduction of any gas. The cap was fitted with two hypodermic needles, one of which contained a rubber plug in the butt of the needle. The anesthetic was injected through the rubber plug as an equal volume of Krebs’ solution was forced out the other needle. To prevent accidental overflow of the injected anesthetic, the tip of the injection port needle projected further into the bottle than the overflow needle. When injecting anesthetics denser than Krebs’ solution, the bottle was held upright so the injected anesthetic fell towards the bottom of the flask, away from the overflow port. On the other hand, the bottle was inverted for injection of ether so that again the injection stream was directed away from the overflow orifice. After the anesthetic had been injected, both needles were plugged with their corresponding styles and the bottle was continuously tipped end to end for two hours so that the glass beads stirred the contents. To produce standard curves, known amounts of anesthetic were assayed to yield a plot of gas chromatograph pen deflection against anesthetic concentration.

Gas Sample Analysis

A 1 ml volume of a gas mixture to be analyzed was drawn very slowly (over about a minute) into a syringe and injected into a gas chromatograph for

### Table 1. Krebs’ Solution—Air Partition Coefficients at 37 C.

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>95 Percent Confidence Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enflurane</td>
<td>12</td>
<td>0.74</td>
<td>0.024</td>
<td>.72 - .75</td>
</tr>
<tr>
<td>Ether</td>
<td>15</td>
<td>11.9</td>
<td>0.62</td>
<td>11.5 - 12.2</td>
</tr>
<tr>
<td>Fluroxene</td>
<td>23</td>
<td>0.81</td>
<td>0.052</td>
<td>.79 - .83</td>
</tr>
<tr>
<td>Halothane</td>
<td>30</td>
<td>0.75</td>
<td>0.076</td>
<td>.73 - .77</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>20</td>
<td>0.55</td>
<td>0.035</td>
<td>.53 - .56</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>13</td>
<td>3.8</td>
<td>0.14</td>
<td>3.7 - 3.9</td>
</tr>
</tbody>
</table>

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1 Composition of Krebs’ solution (mM): Na+, 130; K+, 5.9; Ca++, 2.5; Mg++; 1.22; Cl-, 123; HPO4-2, 1.2; SO4-2, 1.22; HCO3-, 25; plus glucose, 20.8 g/l bubbled with 95 percent oxygen and 5 percent carbon dioxide.
analysis. To establish a calibration curve, a 1-ml syringe was used to inject specific amounts of each anesthetic to be analyzed into bottles of known gas volume (4,000 ml). A ball of aluminum foil was placed in each bottle to provide a means of mixing. A graph of anesthetic concentration against peak height was then constructed from samples of known composition for use as a standard. Appropriate correction was made for pressure-volume changes associated with volatilization of the injected anesthetic.

Partition Coefficient Determination

To determine the solubility of an anesthetic in equilibrium between Krebs' solution and air, the 150-ml flask described above was used. Known amounts of anesthetic were added to 50 ml of Krebs' solution to produce a final partial pressure of about 1 MAC. The flasks were stirred by repeated inversion in a 37°C bath for one hour. For calculation of the partition coefficients, the anesthetic concentrations in the liquid and gaseous phases were then assayed as described above. Recoveries were calculated as a check on internal consistency of the overall assay. Assays were accepted only if the recovery was within 5 per cent of the predicted volume.

Results

Table 1 summarizes the results. The 95 per cent confidence limits are of the order of ±2–3 per cent.

Discussion

The values obtained for Krebs' solution–air partition coefficients averaged 92 per cent (± 2 per cent SE) of the corresponding values reported for water–air solubility. This ratio compares with an average of 94 per cent suggested for saline solution–air coefficients compared with their water–air counterparts.1

References