LIMITATIONS OF THE USE OF POTASSIUM HYDROXIDE-
POTASSIUM CYANIDE MIXTURES IN MANOMETRIC
STUDIES

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The manometric study of biological oxidations by the "direct method"
of Warburg, when carried out in the presence of cyanide, involves the use
of KOH-KCN mixtures (Krebs (4)) for the absorption of carbon dioxide.
The following study describes the rate of distillation of HCN from the ex-
perimental solution under various conditions, and suggests a remedy for
the difficulties occasionally encountered (Machlis (2)) with the above
mixtures.

EXPERIMENTAL

Cyanide was determined by the modified method of Liebig (Kolthoff
and Sandell (3)). To 1.0 ml. of 0.01 M cyanide solution in a test-tube
were added 0.5 ml. of 1.6 \( \times 10^{-3} \) M potassium iodide and 0.5 ml. of 3.0
M ammonium hydroxide. The mixture was then titrated with 2 \( \times 10^{-3} \)
M standard silver nitrate to the first permanent appearance of turbidity.
For the determination of cyanide in concentrations of 0.001 M or less, the
volumes of potassium iodide and ammonium hydroxide solutions were
reduced to 0.3 ml., while the standard silver nitrate solution was diluted
to 4 \( \times 10^{-4} \) M.

Aliquots of a freshly prepared 2.0 M KCN solution were diluted to the
desired concentration by the addition of distilled water either alone or
with sufficient dilute nitric acid to bring the pH to 6.7. Cyanide con-
centrations of the control solutions were then determined by the above
method. 2 ml. of solution (pH 6.7) were added to each of six Warburg
flasks (conical vessels; approximately 16 ml. volume) containing 0.2 ml. of
either a KOH, KCN, or KOH-KCN mixture in the center well, together
with a small square of fluted filter paper (Umbreit et al. (5)). The flasks
were equilibrated for 5 minutes, with shaking, in a 30° bath, following
which the stop-cocks were closed, the first cup removed from the bath,
and 1 ml. of solution taken from the flask for the immediate determination
of cyanide. The remaining vessels were removed from the bath at reg-
ular intervals and cyanide determinations were carried out.

Some loss of cyanide is effected during the adjustment of the pH (Fig.
An additional loss occurs during the short period in which the cyanide solution is transferred to the manometer flasks, and the flasks equilibrated. The latter loss, together with the subsequent disappearance of cyanide, depends upon the nature of the solution in the center well. The use of KOH alone is clearly unsatisfactory for even short time experiments. The distillation of HCN into 10 per cent KOH follows the same course from a 0.001 M solution as from a 0.01 M solution (Fig. 1). In the presence of 10 per cent KOH the pH of the external solution rises from 7.2 at the end of the equilibration period to 8.2 an hour thereafter. With KCN alone in the center well the pH rises from 6.9 to 7.3 during the same interval. The initial pH is 6.7 in all cases.

The concentration of free hydrocyanic acid is 99 per cent of the total cyanide present at pH 7.0 and 87.5 per cent at pH 8.0 (Umbreit et al. (5)). Assuming the rate of distillation of HCN to be a function of its concentration, the curve describing the rate of distillation of HCN from a solution buffered at pH 6.7 would, in the presence of 10 per cent KOH, be slightly steeper than the curve presented in Fig. 1, whereas with KCN in the center well little difference would be observed. At pH values of 7.0 and below the rate of distillation will be independent of pH. At higher pH values the rate of distillation will be directly related to the pH.

Equilibrium conditions were examined in systems with KCN exclusively in the center well and no cyanide in the external solutions. Such systems
represented a simplifying approximation to experiments involving very dilute cyanide solutions. When it was desired to simulate the evolution of carbon dioxide by a tissue, 1 ml. of 1.0 M HNO₃ was added from the side arm to 1 ml. of Na₂CO₃ solution (1 mg. of Na₂CO₃ per ml.; 212 c.mm. of CO₂) in the main chamber. Otherwise, 2 ml. of either distilled water or phosphate buffer were placed directly in the vessel. The center well in each case contained 0.2 ml. of 2 M KCN together with a fluted filter paper.

Table I indicates that the distillation of cyanide from the center well may be appreciable. The extent of this distillation is directly related to the amount of carbon dioxide absorbed. Equilibrium appears to be achieved within approximately 30 minutes. Neither the pH nor the buffer capacity affects the equilibrium under the experimental conditions employed.

### Table I

**Rate of Distillation of HCN from Center Well to External Solution**

External solution initially free of cyanide; 0.2 ml. of 2 M KCN and fluted filter paper in the center well.

<table>
<thead>
<tr>
<th>External solution</th>
<th>Time after equilibration</th>
<th>Cyanide per ml. external solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H₂O</td>
<td>60</td>
<td>2.32</td>
</tr>
<tr>
<td>0.5 M HNO₃</td>
<td>60</td>
<td>2.16</td>
</tr>
<tr>
<td>0.5 &quot; &quot; + 1 mg. Na₂CO₃</td>
<td>30</td>
<td>8.80</td>
</tr>
<tr>
<td>0.5 &quot; &quot; + 1 &quot; &quot;</td>
<td>60</td>
<td>9.52</td>
</tr>
<tr>
<td>0.1 &quot; &quot; KH₂PO₄, pH 7.4</td>
<td>60</td>
<td>2.48</td>
</tr>
<tr>
<td>0.1 &quot; &quot; &quot; 9.0</td>
<td>30</td>
<td>2.20</td>
</tr>
<tr>
<td>0.1 &quot; &quot; &quot; 9.0</td>
<td>60</td>
<td>2.30</td>
</tr>
</tbody>
</table>

The efficiency with which KCN solutions absorb carbon dioxide was investigated. To this end 0.7 ml. of 4 N H₂SO₄ was added from the side arm of a manometer flask to 1 mg. of Na₂CO₃ contained in 2 ml. of distilled water. The released carbon dioxide was absorbed as depicted in Fig. 2. Practically, KCN proved as effective as KOH-KCN mixtures in absorbing carbon dioxide.

A tangent was constructed to the curve defined by solid circles (Fig. 2) at a height corresponding to 80 c.mm. of CO₂ on the ordinate axis. From the slope of this curve at the point of tangency the constant C₁ was derived and, assuming the rate of CO₂ evolution by the system under study to be 300 c.mm. per hour, the following pertinent data were calculated by the

\[ X = R/C (1 - e^{-CT}) \]

where \( X \) equals the volume of CO₂ in the flask at any time, \( R \) equals the rate of evolution of CO₂ by the tissue in c.mm. per minute, \( C \) is a constant, and \( T \) the time in minutes. \( CX \) represents the slope of the curve at any point.
KOH-KCN IN MANOMETRIC STUDIES

method of Dixon and Elliott (1): (1) the time required to achieve equilibrium (in terms of the CO₂ concentration in the gas phase), 33 minutes; (2) the concentration of CO₂ in the gas phase at equilibrium, 36 c.mm.; (3) the error involved by reading the manometer after 10 minutes, 8 c.mm., or less than 3 per cent. For most purposes the error involved

![Graph](http://www.jbc.org/download.png)

**Fig. 2.** The absorption of CO₂ by solutions of potassium cyanide, potassium hydroxide, or mixtures thereof. The curve designations represent the contents of the center well. O, 1.0 M KCN + Dixon paper; ●, 1.0 M KCN + 0.1 M KOH + fluted paper; ○, 1.0 M KCN + 0.1 M KOH + Dixon paper; ●, 10 per cent KOH + fluted paper. Dixon KOH paper was made from 4 sq. cm. of analytical grade filter paper rolled into a cylinder with the upper end cut at right angles and spread to offer the maximum surface.

is very small. When the "first 10 minutes" are included in the equilibration period, the error is negligible.

Fluted papers are as effective as the Dixon KOH papers in effecting rapid absorption. Slivers (5 mm. X 2 cm.) are less satisfactory. The gas uptake in excess of theoretical observed with 10 per cent KOH as absorbent seems to be an oxidation of filter paper as described by Dixon and Elliott.
KOH-KCN mixtures are designed to achieve the same free HCN concentration in the center well as occurs in the external solution. Any addition of KOH beyond the amount necessary for such a condition results in the loss of HCN from the medium (Fig. 1). For most purposes the KCN concentration in the center well is kept at 1.0 M (Umbreit et al. (5)), and the KOH concentration is then varied accordingly, the final volume being 0.2 ml.

The proper mixture to be used with 0.001 M cyanide in the external solution demands an alkali concentration of 0.01 M. The acid-binding capacity of 0.2 ml. of 0.01 M KOH is equivalent to but 22.4 c.mm. of carbon dioxide. Since this capacity is soon exceeded by an actively respiring system, it seems desirable to employ KCN exclusively in the center well and to minimize still further the loss of HCN from the external solution.

The free HCN concentration of a 1.0 M KCN solution, calculated from the hydrolysis constant and the pH of such a solution, is approximately $5 \times 10^{-3}$ M. Thus only at HCN concentrations less than $5 \times 10^{-3}$ M in the external medium will any benefit be derived by addition of KOH to the center well. The purpose of KOH in such instances is to prevent a higher HCN concentration in the center well than in the experimental vessel. Since, in most instances, the KOH present is rapidly utilized in the absorption of CO$_2$, the use of KOH-KCN mixture is indicated only in experiments involving dilute (10$^{-4}$ M or less) cyanide. In the last analysis the choice of a center well solution will depend upon whether it is relatively more hazardous to lose HCN from the external solution to the center well or to exceed the original concentration in the main chamber by distillation of cyanide from the center well. The ability of such mixtures to absorb CO$_2$ must be considered as well as the free HCN concentration. The mixtures suggested by Umbreit et al. to accompany 10$^{-6}$ M and 10$^{-4}$ M cyanide in the external solution do not have sufficient capacity to make efficient absorbing agents.

**SUMMARY**

The rate of distillation of HCN from solution is described during manometric experiments in which either KOH or a KOH-KCN mixture is contained in the center well as an absorbent for CO$_2$. At external cyanide concentrations in excess of 10$^{-4}$ M, the concentration of KOH in a suitable KOH-KCN mixture represents so small a capacity for CO$_2$ absorption as to warrant the initial use of KCN alone. At external concentrations of 0.01 M or greater, any KOH in the center well results in a loss of cyanide from solution. At low cyanide concentration, KOH-KCN mixtures in the center well minimize but do not obviate the back-distillation of cy-
anide from the center well to the external solution. KCN is shown to be a satisfactory absorbent for CO₂, as indicated by studies of the absorption rates.

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