A COLORIMETRIC REACTION OF CHLORIDE ION*

BY FRANCIS BINKLEY

(From the Laboratory for the Study of Hereditary and Metabolic Disorders and the Department of Pathology, University of Utah School of Medicine, Salt Lake City)

(Received for publication, December 9, 1947)

It has been found that the reaction of halogens with certain alkaloids in the presence of persulfate may be used for the determination of chloride, bromide, and iodide ions. In the present report, the determination of chloride will be discussed.

EXPERIMENTAL

Specificity of Reactions— Several alkaloids were tested for their reaction with chloride, bromide, and iodide ions. The test system was as follows: From 0.01 to 0.05 milliequivalent of the halogen compound (sodium salt) was placed in colorimeter cuvettes. Water was added to make a volume of 5 ml. and then 2 ml. of 50 per cent phosphoric acid were added. 1 ml. of a 1 per cent solution of the alkaloid (in 5 per cent phosphoric acid) was added; the solution was mixed and 0.5 ml. of a 1.0 per cent solution of potassium persulfate was added. The tubes were placed in a boiling water bath for 30 minutes, allowed to cool at room temperature for 30 minutes, and diluted to the original volume. The amount of color development was measured at 540 m$\mu$ in a Coleman spectrophotometer, model 6-A. The results with brucine, strychnine, and cinchonidine are summarized in Table I.

From the results given in Table I it is seen that chloride cannot be determined in the presence of bromide or iodide with brucine alone. It is possible, barring interference, to determine chloride in solutions containing no bromide or iodide, to determine bromide in the presence of chloride and in the absence of iodides, and to determine iodides in the presence of chlorides and bromides.

Chloride Method—The method is essentially that given above; a solution of brucine is used but the period of heating is extended to 1 hour. The usable range is from about 0.005 to 0.05 milliequivalent (or from 0.1 to 2.0 mg.) of chloride ion in 5 ml. of sample. With suitable modification and small colorimeter cuvettes, the sensitivity can be increased to 0.01 to 0.1

* These studies were supported by a grant from the United States Public Health Service.
COLORIMETRIC CHLORIDES

mg. in 1 ml. of sample. In Fig. 1 the absorption curve as determined with
the Beckman model DU spectrophotometer is given. The wave-length
of maximum absorption is in the range of 490 m. 540 m has been found
to be a more useful wave-length for measurement. As shown in Fig. 2,
Beer's law is obeyed in the specified range. Theoretical recoveries of

<table>
<thead>
<tr>
<th>Halide</th>
<th>Brucine</th>
<th>Strychnine</th>
<th>Cinchonidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bromide</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Iodide</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

TABLE 1

Approximate Ratios of Color Development of Halogens with Different Alkaloids

Samples of 0.01 milliequivalent of the sodium salts were compared, as described
in the text.

Fig. 1. Absorption spectrum of the color developed in the reaction of 0.004 milli-
equivalent of chloride ion with brucine and persulfate.

Fig. 2. Dependence of color development upon concentration of chloride ion.

chloride were found for cysteine, arginine, lysine, and histidine hydro-
chlorides. Other halogens were the only important sources of interference
encountered.

Determination of Chloride in Plasma or Serum—The method for the
determination of chloride has been adapted to the determination of chloride
in plasma and serum in the absence of bromides. 2 ml. of an ordinary
1:10 tungstate or trichloroacetic acid filtrate were used. The protein precipitant was added to the standards to compensate for contamination with chloride. The colorimetric method was compared with the titrimetric method of Schales and Schales. The colorimetric method proved to be more reproducible than the titrimetric method. In general, however, the colorimetric values were about 2 milliequivalents per liter higher than by the titrimetric procedure. In a series of 100 determinations covering all types of conditions met in the routine laboratory, the colorimetric procedure averaged 1.6 milliequivalents per liter higher than the titrimetric procedure. The details of the clinical application of the method will be presented elsewhere.

**Bromide Method**—If strychnine is used as a color reagent, bromide may be determined in the presence of chloride. In the absence of chlorides, the method in which brucine is used as a color reagent is more sensitive. When strychnine is used, a shorter period of heating (15 minutes) is desirable. The sensitivity is of the same order as for chloride with brucine as the reagent. Little opportunity has been found to apply the method to biological materials but recovery experiments have indicated that bromide in concentrations of from 25 to 200 milliequivalents per liter of plasma may be accurately estimated. In Table II, recoveries of bromide in the presence of chloride are illustrated. These samples were compared with standards containing no added chloride. If chloride was added to the standards to approximate the chloride concentration of plasma, theoretical recoveries were obtained.

**SUMMARY**

Colorimetric methods for chloride and bromide ions have been described. The method for chlorides has been applied to the determination of chloride in plasma in the absence of bromide.

A COLORIMETRIC REACTION OF CHLORIDE ION
Francis Binkley