A rapid colorimetric method for the estimation of urinary chlorides

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SYNOPSIS A rapid and reproducible method for chloride estimation in urine is described. The colour produced is stable and obeys Beer's law over a wide range of concentrations using a simple colorimeter and filter. The method gives good agreement with both the potentiometric titration method of Sanderson (1952) and the mercurimetric titration method of Schales and Schales (1941). The precision of the colorimetric method, though inferior to that of the potentiometric method, is similar to that of the mercurimetric method.

In a review of the available colorimetric procedures for chloride estimation (MacDonald, 1960) it was suggested that the mercuric thiocyanate and ferric chloride methods were the most suitable. The mercuric thiocyanate method appears to be better suited to biological materials containing protein; it was first described by Iwasaki, Utsumi, and Ozawa (1952) and depends upon the fact that mercuric thiocyanate is so feebly dissociated that insufficient thiocyanate ions are liberated to give the characteristic colour with iron (III). On the addition of chloride, however, a soluble mercuric chloride complex is preferentially formed and the thiocyanate ions thus displaced form an orange colour with ferric salts. In aqueous solutions the colour formed does not obey Beer's law, but, when formed in suitable concentrations of dioxan, it gives a linear relation over a wide range of chloride concentrations (Iwasaki, Utsumi, Hagano, and Ozawa, 1956).

The following method has been developed for use with urines; other fluids were measured to test the range of application for the method.

METHODS

1 COLORIMETRIC METHOD For this method the following reagents were made up.

Iron alum solution Ammonium ferric sulphate (AnalaR), 3 g., per 100 ml. in a saturated aqueous solution of sulphamic acid.

Mercuric thiocyanate solution Mercuric thiocyanate 100 mg./100 ml. dioxan (AnalaR): ethanol (9:1).

Chloride standard Dried sodium chloride (AnalaR), 5.38 g. per litre $\equiv$ 100 mEq/l.

Procedure Into a 5 in. $\times$ $\frac{3}{8}$ in. test tube pipette 3.0 ml. of the iron alum solution. Add 0.05 ml. of the sample urine from a 'to contain' pipette. Add 3.0 ml. of the mercuric thiocyanate solution and immediately mix the contents of the tube. Prepare a standard and a reagent blank in the same way using 0.05 ml. of either the chloride standard or distilled water.

Measure the extinction of the tests, the standard, and the reagent blank in an E.E.L. portable photoelectric colorimeter against a water blank using an Ilford 622 filter.

CALCULATION This is done by the equation

$$\frac{\text{Extinction of test} - \text{extinction of reagent blank}}{\text{Extinction of standard} - \text{extinction of reagent blank}} \times 100 = \text{Cl}^- \text{ mEq/l.}$$

2 POTENTIOMETRIC TITRATION This method was used as described by Sanderson (1952) using 0.2 ml. of sample.

3 MERCURIMETRIC TITRATION This method was used as described by Schales and Schales (1941) using 0.2 ml. of sample.

In all methods the same sodium chloride standard ($\equiv$ 100 mEq/l Cl^-) was used.

RESULTS

Using a Unicam SP 700 recording spectrophotometer it was confirmed that the end product formed in the colorimetric method has an absorption maximum at 460 m μ ; the colour was found to be stable for more than one hour at room temperature, and reached the maximum intensity in less than 30 seconds. The final colour intensity was found to increase as the concentrations of either the mercuric thiocyanate or the ferric ammonium sulphate increased, and to decrease with rise in pH.

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The concentrations of mercuric thiocyanate and ferric ammonium sulphate used are chosen to give maximum colour intensity, the mercuric thiocyanate concentration being near to saturation. Sulphamic acid was chosen to give a low pH (0.65) and caused protein to become denatured and soluble. It has been found unnecessary to include a sample blank to allow for pigment interference, in view of the considerable dilution of the sample (approximately 1/100). In this method chloride concentrations between 10 and 160 mEq/l. give a straight line relation with extinction. A non-linear relation is found if the contents of the tube are not mixed immediately.

Chloride estimations have been performed in duplicate on 106 samples by all three methods. Seventy-four urines were chosen so as to test the effect of both high and low chloride concentrations: ileostomy fluids (20), gastric aspirates (11), and bile (1) were included to assess the effect of pigments on the colorimetric method.

DISCUSSION

From the duplicate results obtained by each of the three methods the mean and the standard deviation have been calculated for each method (Table I).

TABLE I
SUMMARY OF RESULTS OBTAINED BY DUPLICATE ESTIMATIONS OF CHLORIDE ON 106 SAMPLES BY EACH OF THREE METHODS USED

Method	Range Encountered	Calculated	
		Mean	Standard Deviation of Method
Mercurimetric	2-155	57.5	2.22
Potentiometric	3-151	57.4	0.94
Colorimetric	2-156	57.7	2.35

All values are expressed in mEq. Cl⁻/l.

From Table II it can be seen that there is no significant difference between the means ($P > 0.02$ in all cases) and that there is good correlation between the three methods ($P < 0.001$). However, the potentiometric method has a significantly smaller variance than either of the other two methods ($P < 0.001$); there is no significant difference between the variance of the colorimetric and mercurimetric methods ($P = 0.2$).

TABLE II
CORRELATION BETWEEN COLORIMETRIC AND TITRIMETRIC METHODS

Methods	Correlation		Comparison of Variances		Comparison of Means	
	r	P	F	P	t	P
Colorimetric/mercurimetric	0.98	<0.001	1.12	0.2	0.83	>0.2
Colorimetric/potentiometric	0.98	<0.001	5.85	<0.001	1.21	>0.2
Mercurimetric/potentiometric	0.99	<0.001	5.23	<0.001	0.42	>0.2

FIG. 1. Comparison of the results obtained with the proposed colorimetric method and the mercurimetric titration method on 106 samples.

FIG. 2. Comparison of the results obtained with the proposed colorimetric method and the potentiometric titration method on 106 samples. ● = urines. ▲ = other biological fluids. The line shown is the calculated functional regression line.

From Figures 1 and 2 it can be seen that the colorimetric method gives good agreement with the two standard methods over the range of chloride concentrations encountered; the line shown in each of these figures is the functional regression line calculated by the method used by Blainey and Squire (1960).

These calculations suggest that the potentiometric method is the most precise of the three methods used, the other two methods being equal to one another in precision. When duplicate estimations are performed all three methods give similar final results. Bile and other pigments do not appear to influence the colorimetric method more than either of the other two methods.

In its present form this method does not appear to be suitable for use with whole serum.

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