A SPECTROCHEMICAL DETERMINATION OF SODIUM IN BLOOD SERUM

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A spectrochemical method for the quantitative analysis of sodium in blood serum has been worked out and used during the past 2 years for routine analyses. It differs in several respects from the methods described by Thomson and Lee (1), Langstroth (2), and Duffendack et al. (3) for measuring sodium in body fluids. The first two require specially constructed sparking arrangements for exciting the sample; the third employs a high voltage A.C. carbon arc. In the present method the regular low voltage D.C. carbon arc equipment is used, with only slight modification (this is manufactured as standard equipment by the Bausch and Lomb Optical Company) and a Bausch and Lomb medium quartz spectrograph. For the sake of completeness and for future reference, the method will be described in detail, although some of the procedures concerning excitation in the low voltage D.C. arc between graphite electrodes are in common use and recently have been discussed by other workers, particularly Cholak (4), Cholak and Story (5), Owens (6), and Pierce et al. (7).

Procedure

An accurately measured volume, 0.5 ml. or less, of serum is diluted 40 times with distilled water. For the internal standard a cadmium chloride solution is prepared containing 1 part in 4000 of cadmium. Equal amounts, 0.25 ml., of these two solutions are placed in a small silica dish and the mixture rapidly ashed on
an electrical heater unit. While still warm, the residue is re-
dissolved in about 0.25 ml. of 10 per cent HCl, transferred by
pipette to the crater of a piece of spectroscopic carbon, and
evaporated to dryness by holding the electrode beside the heater
unit. The use of much larger amounts of HCl causes a serious
loss of sample because of transference into and over the carbon
with repeated filling of the crater.

The carbon electrode is a piece of National Carbon Company
specially pure graphite about 2.5 cm. long, 6.3 mm. in diameter,
with a crater 4 mm. in diameter and 6 mm. in depth. The regular
spectroscopic electrodes of the same company are not suitable,
presumably because of impurities which interfere with the in-
tensity of the sodium line that is used for measurement. The
electrode is connected to the negative side of the power line and
is placed below the positive electrode, which is a 3.5 cm. piece of
the regular variety of graphite. The positive electrode also has
a crater which keeps the discharge from running up the outside
of it. A simple attachment is provided for the Bausch and Lomb
arc stand to hold the electrodes in line at an angle of about 15°
with the vertical, the upper electrode being nearer the spectro-
graph. The negative electrode containing the sample may be
used again for a second sample, the positive electrode four times.
The arc is supplied by a 115 volt d.c. generator. The current is
initially adjusted to read 6 to 7 amperes by means of the series
resistor. A fresh electrode is burned for 20 seconds before the
sample is introduced and for 75 seconds while the sample is
arced and the photographic exposure made.

The light from the negative electrode and its immediate neigh-
borhood is projected in an approximately parallel beam onto the
slit of the spectrograph by means of a 7.6 cm. focal length quartz
plano-convex spherical lens. The arc is 60 cm. from the slit.
With appropriate separation of the electrodes, the light from the
positive electrode falls below the slit. As the hot spot moves
around the rim of the crater of the lower carbon, the inclination
of the electrodes prevents the light emitted in the direction of the
optical axis of the spectrograph from being cut off by the electrode
itself. The width of the slit is 0.01 mm. In front of the slit is
placed a rotating sector consisting of a half cylinder milled in a
steel rod 9.5 mm. in diameter. This arrangement constitutes an
adaptation of the method for measuring relative intensities described by Hasler and Lindhurst (8) for use with a grating spectrograph. The spectrum lines that are measured in the analysis of sodium are the unresolved pair of sodium lines of 2680.3 and 2680.4 Å and the cadmium line of 2677.6 Å. The background intensity in this region is not troublesome. By means of an adapter, a short piece of 35 mm. positive motion picture film is inserted in the plate holder, covering only the region of measurement, and two exposures are made. The photographic processing is done in the usual way.

*Interpretation of Spectrum Plate*

The half cylinder sector produces a spectrum line which decreases in density from both ends toward the center, so that there are two extinction points whose separation depends on the intensity of the light striking the photographic emulsion. The distance $L$ between the two extinction points is measured by means of a Bausch and Lomb spectrum-measuring magnifier. For this type of sector the intensity of a spectrum line in the source is inversely proportional to the distance between the two extinction points for small values of the ratio $L:2R$, where $2R$ is the diameter of the sector. For the quantities of normal serum and cadmium solutions given above, the spectrum lines are of nearly the same intensity and $L$ is about 1.5 mm.

The working or calibration curve is shown in Fig. 1. In the construction of this curve a solution of NaCl, KCl, CaCl₂, MgCl₂, and NaH₂PO₄·H₂O was prepared equivalent in inorganic content to normal serum, and the ashing and arcing procedures mentioned above were carried out. The value of sodium concentration in normal human serum was taken as 335 mg. per 100 ml. of serum. Shohl (9) gives the preferred value as 330 mg. per 100 ml. of serum, but it is immaterial as far as the calibration curve is concerned which value is used. The NaCl content was then changed to give the sodium values listed along the axis of abscissas. Each point is an average of eight like determinations.

The possible uncertainty in the reading of $L$ is about ±0.05 mm., for $L = 1.5$ mm., so that the determination of a ratio of intensities is accurate to ±5 per cent. The error is substantially the same for a 50 per cent increase or decrease in intensity. The
error in an intensity determination introduced because of the appreciable magnitude of $L:2R$ is less than 0.2 per cent. Larger fluctuations in intensity appear, however, because of variations occurring in the process of ashing and arcing. These fluctuations

\[ \text{Table I} \]

Duplicate Analyses of Serum for Sodium by Chemical and Spectrochemical Methods

The results are expressed in mg. of sodium per 100 ml. of serum.

<table>
<thead>
<tr>
<th>Spectrographic analysis</th>
<th>Chemical analysis</th>
<th>Per cent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>312</td>
<td>304</td>
<td>-2.5</td>
</tr>
<tr>
<td>313</td>
<td>321</td>
<td>+2.5</td>
</tr>
<tr>
<td>332</td>
<td>322</td>
<td>-3.0</td>
</tr>
<tr>
<td>328</td>
<td>327</td>
<td>-0.3</td>
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<tr>
<td>302</td>
<td>307</td>
<td>+1.6</td>
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<tr>
<td>291</td>
<td>280</td>
<td>-3.7</td>
</tr>
<tr>
<td>305</td>
<td>311</td>
<td>+2.0</td>
</tr>
<tr>
<td>298</td>
<td>297</td>
<td>-0.3</td>
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</tr>
<tr>
<td>299</td>
<td>296</td>
<td>-1.0</td>
</tr>
</tbody>
</table>

Average.............................................. -0.7
in intensity are random in character. The standard deviation for a series of 80 determinations on like samples of serum was found to be ±8 per cent. In practice, to obtain a measurement of sodium with a standard error not greater than ±3 per cent, it is customary to make six determinations and compute the average. These may be done in 1 hour. As a comparison between this spectrochemical method and a well known chemical method, the analyses of a number of serum samples from fever patients are presented in Table I. The chemical analyses were made by Dr. H. E. Thompson, Jr., of the Department of Obstetrics and Gynecology, using the uranyl zinc acetate method of Barber and Kolthoff (10) as modified by Salit (11). The agreement is within the standard error, about ±3 per cent for both methods.

DISCUSSION

The spectrochemical method described here is of a general nature and is adaptable to the measurement of sodium in other biological materials. The conditions for maximum sensitivity were sought in order to give a method requiring a minimum of material. The influence on the sodium-cadmium ratio of positive elements other than sodium would have to be investigated in each case. However, for blood serum the other elements are in such relatively small proportions that the curve shown is the same, in the region of normal sodium, when only sodium and cadmium are present in the test sample. Small amounts of hemolysis also do not affect the measurement. Furthermore, the method may be simplified for serum by omitting the ashing, and introducing a mixture of the serum and cadmium solutions directly into the electrode. The precision of the method is thereby somewhat increased and the time for an analysis materially reduced.

Several other experiments were carried out with test solutions to determine the validity of the method of measurement under various conditions differing considerably from those discussed above. The intensity ratio for two values, 1.00 and 1.84, was found to be the same before and after reducing by 53 per cent the intensity of the light from the arc by means of a wire screen placed over the projection lens. This result shows that the whole procedure of measuring relative intensities, involving sector,
photographic emulsion, processing, and measuring by eye is in practice reliable over a good range of intensities. The ratio of intensities is independent of a 50 per cent increase in the arc current. If the amounts of sodium and cadmium in a sample are uniformly increased or decreased, the ratio of intensities remains the same. The intensity ratio is about equally sensitive to changes in cadmium as to changes in sodium. All of these results are established of course only within the experimental error. The intensities of the sodium and cadmium lines in themselves, however, are mutually dependent in some degree on the absolute amounts of each in the arc, and the calibration curve shown does not necessarily represent the variations in sodium line intensity with quantity of sodium in the arc. Also, the calibration curve and some of the results described above concerning conditions in the arc may be somewhat different if other sodium or cadmium lines are chosen for measurement.

SUMMARY

A spectrochemical method for the quantitative analysis of sodium in blood serum is described which, for the most part, requires only commonly used spectrographic equipment and laboratory facilities. A determination with a standard error of ±3 per cent may be made in 1 hour on a sample of 0.5 ml. or less. The useful range is from 5 to 50 \( \gamma \) of sodium. The method is applicable to the measurement of sodium in other biological materials.

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