Investigation of a Potential Source of Difficulty in the Use of the AutoAnalyzer

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An investigation of the deleterious action of alkaline ferricyanide on the glass heating coil of the AutoAnalyzer showed not only an increase in fragility of the coil with use but a substantial increase in coil volume, a change which can cause standard readings to exceed acceptable limits.

The passage of a chemical mixture through a glass coil immersed in a temperature-controlled-liquid bath is employed as a part of the AutoAnalyzer system in order to accelerate and control the rate of certain chemical reactions. Methodology (1-4) for this instrument employs chemical reactions which may or may not go to completion during the passage through the constant-temperature heating bath. Variations in the volume capacity of this glass coil might induce unaccountable variations in the incubation time, resulting in unexplainable shifts in the standard curve and necessitating rather major reagent adjustments in order to obtain acceptable calibration curves.

Our attention was first centered on the problem of the heating-bath module when it was noted that certain of the glass coils became extremely fragile after long continuous use. Upon inspection, we found that the wall thickness of one broken glass coil was approximately 1.0 mm. at the inlet and less than 0.3 mm. at the broken area near the center of the coil. We noted that from one coil to another it was often necessary to adjust radically the concentration of the reagents for the diacetyl monoxime method for urea estimation in order to obtain a satisfactory calibration curve. These observations led us to suspect that some reaction was taking place which was enlarging the I.D. of the glass tubing, thereby weakening the glass wall and possibly increasing the total volume of liquid contained in the heating coil.

In order to check for any variation in the volume capacity of our
coils, we chose 2 new coils and 3 coils that had been in service for varying periods of time. We confined our attention to the 1.6-mm. $\times$ 40-ft. coils. The volume of each coil was determined by connecting a 50-ml. syringe through Tygon sleeving to one outlet of the empty glass coil. Water was forced into the coil from the syringe and the volume recorded when water appeared at the outlet on the opposite end. The results of this determination are shown in Table 1.

Transit time through the coil was determined by placing 3 manifold tubes on the multichannel proportioning pump, 0.100 size for reagent, 0.020 size for sample, and 0.056 size for air, to give a flow rate of approximately 4.76 ml./min. These manifold tubes were connected through a "cactus" fitting to the inlet side of a heating-bath module. The outlet from the heating-bath module was connected to a colorimeter equipped with a 15-mm. flow cell in order to furnish the same back pressure normally found in the controlled-temperature module. Water was pumped through the reagent and sample manifold tubing until a stable flow and bubble pattern were observed. The sample tube was then inserted into a bottle of red ink for 4 sec. and then returned to the water container. By means of a stop watch and visual observation, the time required for the sample to pass through the coil was easily determined. This procedure was repeated for each of 5 coils with the heating-bath temperature at 22° and 95°. The results of this experiment are given in Table 1.

On June 2, 1965, the volume capacity of 2 new heating coils, 1 an inner and 1 an outer coil, was determined and found to be 23 ml. each. These coils were mounted in a heating-bath module and placed in operation as part of a dual assembly performing glucose determinations on a schedule of 24 hr./day, 7 days per week. The alkaline ferricyanide method (5) was employed. During the ensuing time period, the instruments were stopped only for short periods for minor maintenance. After 32 days of essentially continuous operation, the heating-bath module was removed.

The volumes of the bath coils were checked at this time and found to

<table>
<thead>
<tr>
<th>Coil No.</th>
<th>Position</th>
<th>Condition</th>
<th>Total Volume (ml)</th>
<th>Transit time at 22°</th>
<th>Transit time at 95°</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Outer</td>
<td>New</td>
<td>23</td>
<td>5 min. 23 sec.</td>
<td>2 min. 29 sec.</td>
</tr>
<tr>
<td>2</td>
<td>Inner</td>
<td>New</td>
<td>23</td>
<td>5 min. 22 sec.</td>
<td>2 min. 31 sec.</td>
</tr>
<tr>
<td>3</td>
<td>Outer</td>
<td>Used</td>
<td>31</td>
<td>7 min. 10 sec.</td>
<td>3 min. 15 sec.</td>
</tr>
<tr>
<td>4</td>
<td>Inner</td>
<td>Used</td>
<td>36</td>
<td>8 min. 34 sec.</td>
<td>3 min. 54 sec.</td>
</tr>
<tr>
<td>5</td>
<td>Inner</td>
<td>Used</td>
<td>49</td>
<td>11 min. 29 sec.</td>
<td>5 min. 28 sec.</td>
</tr>
</tbody>
</table>

*Heating coils were all 1.6 mm. $\times$ 40 ft.
be 34 ml. It is our assumption that the alkaline ferricyanide reagent employed in this determination had reacted with the glass and dissolved the inner wall, thereby increasing bath coil capacities by 11 ml.

The effect of such variations in coil capacity on the ferricyanide method for glucose and the diacetyl monoxime method for urea were investigated. The instrument was set up with a new glass coil having a 23-ml capacity. After an acceptable standard curve was obtained, another constant-temperature module containing a 31-ml capacity coil was substituted in the system and a second standard curve obtained. A third constant-temperature module having a coil capacity of 36 ml was substituted and a third standard curve was run.

An increase in transmission of 3% was found in the glucose standards above 150 mg./100 ml. as a result of increasing the coil volume from 23 ml to 36 ml. This increase is insufficient to necessitate a reagent alteration and is well within the day-to-day variations found in this determination. It was noted, however, that in the coil with the largest volume, there was a greater tendency for the exit stream to move in an intermittent flow pattern. We have termed this type of movement a "surging" flow.

In the urea method, the per cent transmission (%T) for a given standard varies markedly with changes in coil volume, as is shown in Fig. 1. For a urea standard containing 70 mg./100 ml., the change is

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**Fig. 1.** Changes in urea standard curve as result of differences in coil volume. A indicates curve produced by coil with 23-ml volume; B, 31 ml.; and C, 36 ml. First 2 peaks are 10-mg. standard; third, 30-mg. standard, fourth, 50-mg. standard; fifth, 70-mg. standard; sixth, 100-mg. standard; seventh, 150-mg. standard; and eighth, 200-mg. standard.
approximately 1% T for each 1-ml. change in coil volume. As the concentration of urea in the standards was either increased or decreased from 70 mg./100 ml., sensitivity to changes in coil volume decreased. In our laboratory, one of the criteria for an acceptable urea standard curve is that the 150 mg./100 ml. standard fall between 5% T and 15% T. Increased sensitivity of the urea reaction as a result of leached coils can cause the 150 mg./100 ml. standard reading to exceed these limits.

The possible action of other chemical solutions on the glass coils was not tested; nor was the effect of increased incubation time on other determinations investigated.

In those determinations where incubation at constant temperatures for a constant time is important, the unexpected introduction of a different incubation time may result in a dramatic shift in the standard curves. Such sudden shifts can be most disconcerting in a well-controlled laboratory. We recommend that coils which are being used for the ferricyanide method of glucose estimation be marked so that they may be identified when removed for maintenance and cleaning. Periodic checks on the capacity of these coils and a rotation program when the capacity increases to a given volume may prevent rupture of a weakened glass wall at a particularly crucial time.

References