Part at least of the error in the method can be attributed to the presence of p-hydroxybenzaldehyde, the light absorption curve of which is close to that of vanillin. For this reason Pisano recommended measuring vanillin at a wavelength at which the interference due to p-hydroxybenzaldehyde is minimal. However, this procedure is not entirely satisfactory since the amount of p-hydroxybenzaldehyde present may sometimes be very greatly in excess of that of vanillin, because of a high rate of excretion of a precursor, p-sympatol (N-methyl-β-(p-hydroxyphenyl)-ethanolamine).

Although the Pisano method is not in use in these laboratories, we have had occasion to study p-sympatol, initially because of its interference in the chromatographic detection of metanephrine. We have now found it to be present in relatively large quantity in orange squash or fresh orange juice, from which it may readily be isolated by absorption on Dowex-50, elution with ethanolic NH₃ and paper chromatography. The amine appeared indistinguishable from authentic p-sympatol in its chromatographic behaviour and reactions (Coward, Smith and Wilson, 1964) and gave p-hydroxybenzaldehyde on periodate oxidation. Heavy consumption by hospital patients in particular of fruit squashes, renders it desirable for users of Pisano’s method to investigate the p-sympatol content of these and similar comestibles, and to ensure the imposition of adequate dietary restrictions in order to improve the specificity of the determination.

The excretion of p-sympatol has often been observed in these laboratories to be increased in subjects during periods of exposures to stress. The known pharmacological properties of the amine and its close structural relationship to adrenaline rendered attractive the possibility that it might be of fundamental physiological importance. This hypothesis was difficult to reconcile with the wide normal range of excretion and may now be discarded. We are now of the opinion that stress may induce changes in the metabolism of dietary p-sympatol. The normal metabolism might prove an interesting field for study since despite wide variations in the intake of the amine, its expected metabolite, p-hydroxymandelic acid, is excreted in relatively constant amounts (Kakimoto and Armstrong, 1962).

Addendum—Since this manuscript was submitted, a detailed report on the occurrence of p-sympatol in citrus plants has been published (Stewart et al. 1964).

References

The Use of Ultramicro Technique in the Clinical Laboratory
No. 3. The Determination of Alkaline Phosphatase

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The method for the determination of alkaline phosphatase which is described has been adapted to the Sanz ultramicro analytical system from King and Kind’s aminophenazone technique.

**Principle.** The enzyme phosphatase liberates phenol from the substrate, disodium phenylphosphate. The liberated phenol forms a red coloured quinone compound with 4-aminophenazone in the presence of an oxidising agent, acting in alkaline conditions. In the case of alkaline phosphatase determination, the hydrolysis is performed at pH 10.0.

The presence of protein does not affect the reaction and therefore the test may be carried out without protein precipitation.

**Reagents.**
1. Buffer. M/10 sodium carbonate – bicarbonate: 6.36 g. anhydrous sodium carbonate and 3.36 g. sodium bicarbonate are dissolved in water and the volume made up to one litre.
2. Substrate. M/100 disodium phenylphosphate: 2.18 g. disodium phenylphosphate
dissolved in water and made up to one litre. This reagent is stored in the refrigerator and preserved with a few drops of chloroform.

3. Stock phenol standard. 100 mg./100 ml.
4. Working phenol standard. 25 mg./100 ml. The working standard phenol is prepared fresh daily by diluting 25 ml. of stock standard to 100 ml. with distilled water.

5. 0·5N. sodium hydroxide.
6. 0·5M. sodium bicarbonate.
7. 0·6% 4-aminophenazone in distilled water.
8. 2·0% potassium ferricyanide in distilled water.

Reagents (7) and (8) are prepared at weekly intervals.

All chemicals are A.R. quality except the 4-aminophenazone. The reagents which are used in the ultramicro technique are essentially the same as those used in the aminophenazone method of King and Kind. A stronger phenol standard is used and the concentration of the potassium ferricyanide has been slightly modified to produce a better colour reaction.

**Apparatus.** The ultramicro modification required the preparation of a set of semi automatic reagent and sample pipettes. The manufacture of these pipettes has been described previously. (Macfarlane 1963)

1. 'Reagent' pipette, 100 microlitres for buffer.
2. 'Sample' pipette, 100 microlitres for measuring substrate and distilled water.
3. 'Sample' pipette, 20 microlitres for measuring serum and standard.
4. 'Reagent' pipette, 80 microlitres for sodium hydroxide.
5. 'Reagent' pipette, 120 microlitres for sodium bicarbonate.
6. 'Reagent' pipette, 100 microlitres for 4-aminophenazone.
7. 'Reagent' pipette, 100 microlitres for potassium ferricyanide.

**Procedure.** The test is performed in small glass test tubes of 2 ml. capacity to allow ample space for thorough mixing between additions.

Prepare 'Test', 'Test Blank', 'Standard' and 'Reagent Blank' tubes as follows

1. Measure 100 microlitres of buffer into each of the four tubes.
2. Measure 100 microlitres of substrate into the tubes labelled 'Test' and 'Test Blank' and, using the same pipette, measure distilled water into the tubes labelled 'Standard' and 'Reagent Blank'. Using the pipette in this way, ensures that the final volumes of tests, blanks and standards are identical.
3. Measure 20 microlitres of serum into the tube labelled 'Test', mix the contents and cap the tubes with parafilm. Place the tube in a rack in the water bath at 37°C for 15 minutes. While the tests are incubating and using the same pipette, prepare the 'Standard' and 'Reagent Blank' by measuring 20 microlitres of working standard phenol and 20 microlitres of distilled water into the appropriate tubes.
4. After the incubation of the 'Test', add 80 microlitres of sodium hydroxide to all of the four tubes. Prepare the 'Test Blank' by adding 20 microlitres of serum to the tube labelled 'Test Blank'.
5. Complete the colour development by adding 120 microlitres sodium bicarbonate, 100 microlitres 4-aminophenazone and 100 microlitres of potassium ferricyanide. The contents of each tube are well mixed between each addition.

The red colour which is produced must be read within ten minutes in the spectrophotometer at 510 mμ setting to zero with water.

**Calculation**

\[
\frac{\text{O.D. Test} - \text{O.D. Test Blank}}{\text{O.D. Standard} - \text{O.D. Reagent Blank}} \times 25 = \text{King-Armstrong units per 100 ml.}
\]

A King-Armstrong unit is defined as the amount of the enzyme which will liberate 1 mg. of phenol from disodium phenyl phosphate in 15 minutes at 37°C and hence units per 100 ml. —mg. of phenol liberated.

**Results.** Twenty random samples of serum were analysed by the proposed ultramicro technique and by King and Kind's aminophenazone micro-method. Comparison of the results obtained by the two methods is shown in Table 1 and statistical analyses reveals no significant variation.

Twenty replicate analyses were performed on each of three samples of serum with different
TABLE I

<table>
<thead>
<tr>
<th>King and Kind aminophenazone method</th>
<th>Ultramicro method</th>
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</thead>
<tbody>
<tr>
<td>(K.A. units/100 ml.)</td>
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<td>9.2</td>
<td>9.9</td>
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<td>11.7</td>
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</table>

\[ t = -28.5 \text{ } 0.8 > P > 0.7 \]

Comparison with King and Kind's aminophenazone micro method.

Conclusion. The ultramicro modification which is presented gives results which are in close agreement with King and Kind's aminophenazone micro method.

The advantages of the ultramicro technique are economy of serum and the simplicity of technique achieved by the preparation of a set of reagents with micro-pipettes attached.

References


TABLE II

<table>
<thead>
<tr>
<th>Mean Alkaline phosphatase value (units/100 ml.)</th>
<th>Serum No. 1</th>
<th>Serum No. 2</th>
<th>Serum No. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.2</td>
<td>22.5</td>
<td>47.5</td>
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<tr>
<td>Range</td>
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<td>22 – 23.2</td>
<td>44 – 50</td>
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<tr>
<td>S.D.</td>
<td>0.45</td>
<td>0.25</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Alkaline phosphatase values of 20 replicate analyses on each of three samples of serum.

BOOK REVIEWS

"Biochemical Problems of Lipids" B.B.A. Library—Volume I. Edited by A. C. FRAZER. Elsevier 130s.

This book contains the full text of some fifty-four papers with the discussion given at the seventh International Conference of Biochemical Problems of Lipids held in Birmingham in July, 1962. The main theme of the papers is the absorption of lipids. There are six sections in the book.

The first deals with intraluminal aspects of fat absorption and is concerned mainly with the function of bile. There are also two papers concerned with bacteria and faecal fat.

The second deals with the structural aspects of the intestinal cell and absorption studies. The