Letter to Editor

Vitamin B12 Binding by Intrinsic Factor

Shum, O'Neill, and Streeter (1971) draw some fairly far-reaching conclusions from their findings of the effect of pH on vitamin B12 binding.

One important factor which they have not considered is the effect of pH on albumin-coated charcoal. They assume that the critical ability of coated charcoal to discriminate between bound and free vitamin B12 is preserved throughout the pH range they have employed in their investigation.

Shum and his co-workers specify the discrepancy between their results and those of McGuighan (1967) and observe that 'it is not apparent whether a genuine inconsistency exists'. McGuighan investigated vitamin B12 binding by gastric juice and extracted the free vitamin by membrane dialysis. He found no decline of vitamin B12 binding with falling pH. Furthermore Hippe and Olesen (1971) have shown that the free energy of reaction for cyanocobalamin and intrinsic factor hardly fluctuates between pH 2 and 10. Our own test systems were somewhat less sophisticated, though possibly the results are more easily understood.

In preliminary studies concerned with release of blocking antibody from intrinsic factor at acidic pH (Rose and Chanarin, 1969; Rose, 1970) our findings were similar to those of McGuighan (1967) and conflicted with the findings of Shum, O'Neill, and Streeter (1971) and of Goldberg and Fudenberg (1969).

Rates of Combination of Intrinsic Factor with Vitamin B12

COMBINATION OF NORMAL GASTRIC JUICE WITH VITAMIN B12 AT NEUTRAL pH

Ten ml of neutralized normal gastric juice was introduced into 5 ml of buffered saline at 4°C. The vitamin B12-binding capacity of this gastric juice had been previously tested and was about 70 ng/ml. Five ml of 67cobalt vitamin B12 (200 ng/ml) was rapidly dispensed into the gastric juice. The total mixture thus contained 700 vitamin B12 binding units and 1,000 ng of vitamin B12. This was mixed and 1 ml aliquots were removed with a Summit spring-loaded syringe and delivered at stated intervals into glass bottles containing serum-coated charcoal in saline. The coated charcoal adsorbed the unbound vitamin B12, thus curtailing the combination of gastric juice binders with vitamin B12. The suspension was shaken for two minutes, centrifuged at 3000 rpm for 10 minutes, and the clear supernatant was decanted into calibrated tubes for radioactive counting.

THE COMBINATION OF NORMAL GASTRIC JUICE WITH VITAMIN B12 AT pH 3.2

A similar experiment was carried out to test the combining rate of normal gastric juice with vitamin B12 at pH 3.2. Ten ml of normal gastric juice was added to 5 ml of 0.2 molar phosphate-citrate buffer at pH 3.2, and 67cobalt vitamin B12 was added as described above. One ml aliquots were transferred at stated intervals into a suspension of serum-coated charcoal in 0.2 M disodium hydrogen phosphate. The suspension was shaken, centrifuged, and decanted as itemized above.

Results

The results of a typical experiment are shown:

<table>
<thead>
<tr>
<th>Time (Sec)</th>
<th>B12 Bound (ng)</th>
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<tbody>
<tr>
<td></td>
<td>pH 7.0</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
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<tr>
<td>10</td>
<td>42</td>
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<td>15</td>
<td>46</td>
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<td>44</td>
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<tr>
<td>50</td>
<td>44</td>
</tr>
<tr>
<td>60</td>
<td>44</td>
</tr>
<tr>
<td>10 min</td>
<td>47</td>
</tr>
</tbody>
</table>

Vitamin B12 bound per ml of incubation mixture

Comment

There was no significant difference in the rate or order of combination between normal gastric juice and vitamin B12 at pH 7.5 and pH 3.2.

Since many of the procedures itemized by Shum, O'Neill, and Streeter (1971) employ charcoal adsorption it is certainly possible that unbuffered systems are susceptible to pH effects. No information is provided in their paper concerning the pH in the tests described. For instance, in the radioisotope dilution method of measurement by Raven, Robson, Walker, and Barkham (1969) for measurement of serum vitamin B12 levels, the pH of the extract after autoclaving serum plus hydrochloric acid is 1.6. In the radioimmuno assay of Ardeman and Chanarin (1963) for intrinsic factor and for type II antibody to intrinsic factor the pH remains between 7 and 8.

The conclusions which the authors draw concerning the sequence of events involved in physiological vitamin B12 binding by intrinsic factor seem questionable. A possible explanation is that their findings are due to an adsorption artefact.

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References


