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 B₁₂COMBINATION 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 ⁵⁷cobalt 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 ⁵⁷cobalt 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:

Time (Sec)	B ₁₂ Bound (ng)	
	pH 7.0	pH 3.2
5	40	38
10	42	40
15	46	44
20	45	44
30	43	44
40	44	44
50	44	44
60	44	41
10 min	47	47

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

Raven, Robson, Walker, and Barkhan (1969) for measurement of serum vitamin B₁₂ levels, the pH of the extract after autoclaving serum plus hydrochloric acid is 1.6. In the radioimmune 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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