

coated charcoal and was 635 pg by using type I coated charcoal. The variation of results (from -15% to +9%) between the untreated coated charcoal (type I) and the treated coated charcoals (type II and type III) is neither large enough nor systematic enough to contradict our previous results (Shum, O'Neill, and Streeter, 1971a) where we showed, at extreme pH values, reductions in the bound vitamin B₁₂ values of greater than 90%. In order to relate these results to the pH treatment of the coated charcoal, we expressed the bound ⁵⁷Co-B₁₂ in each tube as a percentage of the highest value of bound ⁵⁷Co-B₁₂ in each series. These results are presented in the Figure. It can be seen that, in the pH range of 1.2 to 12.0, there is no appreciable difference in the amount of bound ⁵⁷Co-B₁₂ in both series, using either type II or type III coated charcoal.

COMMENT

The evidence presented here shows that albumin-coated charcoal previously exposed to extreme pH conditions (type III coated charcoal) retains its ability to differentiate free vitamin B₁₂ from bound vitamin B₁₂. This ability of the coated charcoal is also preserved even when it is kept at extreme pH conditions (type II coated charcoal) until it is added to the vitamin B₁₂ solution. There seems little support from these observations to suggest that pH has any significant effect on albumin-coated charcoal.

Indeed, our previous article (Shum *et al*, 1971a) contained a section referring to the binding of vitamin B₁₂ by non-intrinsic factor binders in human gastric juice. We found no evidence that this binding was affected by pH changes. Yet if our results

were erroneous due to an unexpected effect of pH extremes on the albumin-coated charcoal then both intrinsic factor and non-intrinsic factor binders should have shown similarly shaped binding curves.

In view of our previous work and the results presented here, we repeat our suggestion that the *in vitro* binding of vitamin B₁₂ by intrinsic factor is pH dependent. Between pH 6.5 and 10 the binding capacity is maximal and is fairly constant; outside this pH range the binding capacity decreases and drops sharply to about 10 to 15% of the maximum at pH below 2 or above 12.2. Recently, we have also reported similar observations on the pH effect of the binding of vitamin B₁₂ by the salivary vitamin B₁₂ binder (Shum, Bandouvakis, Newman, and O'Neill, 1972).

HING-YAN SHUM
BARRY J. O'NEILL
ARTHUR M. STREETER

*Division of Haematology
Repatriation General Hospital
Concord, NSW, Australia*

References

Rose, M. S. (1971). Vitamin B₁₂ binding by intrinsic factor. Letter to the Editor. *J. clin. Pathol.*, **24**, 771.
Shum, H. Y., Bandouvakis, J., Newman, D. C., and O'Neill, B. J. (1972). Studies on the binding of vitamin B₁₂ by human saliva. *Pathology*, **4**, 145-151.
Shum, H. Y., O'Neill, B. J., and Streeter, A. M. (1971a). Effect of pH changes on the binding of vitamin B₁₂ by intrinsic factor. *J. clin. Pathol.*, **24**, 239-243.
Shum, H. Y., O'Neill, B. J., and Streeter, A. M. (1971b). A modified method for assaying intrinsic factor blocking antibody in serum. *Pathology*, **3**, 311-315.

Ortho Fibrindex as a Source of Thrombin

Further to the recent correspondence on the use of Ortho Fibrindex as a source of thrombin, we too have found that this reagent is unsatisfactory when used in other test systems.

When Ortho thrombin is used to process serum samples before assay, the resultant FDP assay is far higher than when the same sera are treated with other commercially prepared thrombins (Maw's, Parke Davis, Hoechst).

If Ortho thrombin is used in the performance of the euglobulin clot lysis time, abnormally short lysis times occur (5-6 minutes).

In addition to Maw's and Parke Davis thrombin, we have found Hoechst thrombin to be equally satisfactory, with the advantage that it is supplied in 60-unit lyophilized quantities.

In the course of recent correspondence with Ortho Diagnostics, they have acknowledged that the human thrombin marketed by them does in fact contain contaminants, but maintain that their product is satisfactory when used in accordance with their directions.

S. A. LIGHTOWLER
J. MARBLE
Walsgrave Hospital
Coventry