

## Letters to the Editor

### Effect of pH on Albumin-coated Charcoal

Using a charcoal adsorption technique, we recently produced evidence that the *in vitro* binding of vitamin B<sub>12</sub> by intrinsic factor was pH dependent (Shum, O'Neill, and Streeter, 1971a). Rose (1971) questioned our findings and suggested that our results may have been due to an impairment of the ability of albumin-coated charcoal to discriminate free vitamin B<sub>12</sub> from bound vitamin B<sub>12</sub> at extreme pH values. No direct evidence was presented to support this suggestion and we ourselves have not had the opportunity to reply until some time after the criticism was published. In our article, we showed that albumin-coated charcoal adsorbed 98-99% of the free vitamin B<sub>12</sub>, irrespective of pH. We wish to present evidence that the ability of albumin-coated charcoal to differentiate free vitamin B<sub>12</sub> from bound

vitamin B<sub>12</sub> is not impaired in the pH range 1.2 to 12.0.

#### TREATMENT OF COATED-CHARCOAL

##### Type I

This albumin-coated charcoal was prepared as described by Shum, O'Neill, and Streeter (1971b).

##### Type II

Aliquots of the type I coated charcoal in separate tubes were centrifuged, the supernatants decanted and made up to their initial volumes with solutions of pH values between 1.2 and 12.0. After mixing, these suspensions of coated charcoal were allowed to stand at room temperature for about 20 minutes before use.

##### Type III

A separate batch of type II coated-charcoal suspension was prepared as above. After the 20-minute incubation period, the coated-charcoal suspensions were centrifuged again, the supernatants decanted and each tube made up to its initial volume with distilled water.

#### ABILITY OF TREATED COATED CHARCOAL TO DIFFERENTIATE FREE VITAMIN B<sub>12</sub> FROM BOUND VITAMIN B<sub>12</sub>

Of diluted human gastric juice (total vitamin B<sub>12</sub> binding capacity about 0.6 ng/ml) 0.5 ml was added to a series of tubes, each containing 1.0 ml <sup>57</sup>Co-B<sub>12</sub> (1.2 ng/ml) and 5.0 ml Sorensen's phosphate buffer (pH 7.4). After mixing and incubation at room temperature for 30 minutes, 2.0 ml of each suspension of type II and type III coated charcoals were added to separate tubes. The tubes were then centrifuged at 3000 rpm for 20 minutes and an aliquot of the clear supernatant was taken for radioactivity counting. Control tubes were treated in an identical manner except that type I coated charcoal was used. The amount of <sup>57</sup>Co-B<sub>12</sub> bound in each tube was then calculated by relating its supernatant radioactivity to the radioactivity of a standard tube which contained 1.0 ml <sup>57</sup>Co-B<sub>12</sub> (1.2 ng/ml) and 7.5 ml distilled water. These results varied from 539 to 604 pg by using type II coated charcoal from 632 to 692 pg by using type III

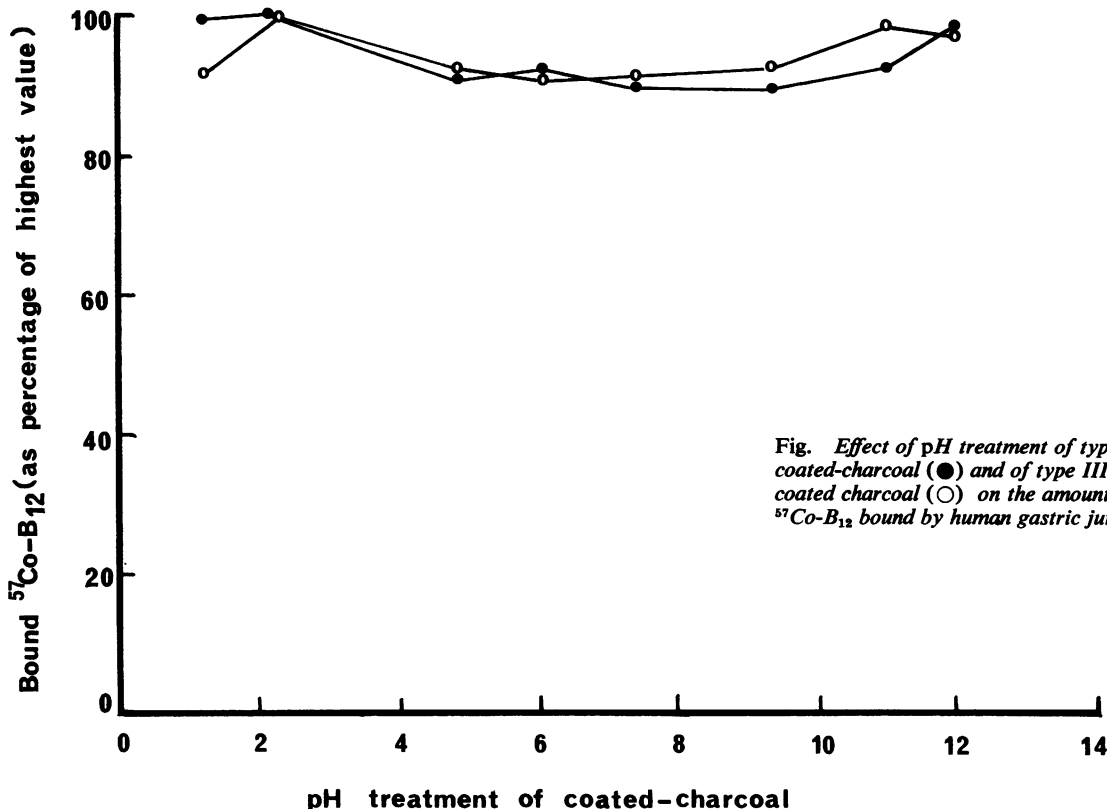


Fig. Effect of pH treatment of type II coated-charcoal (●) and of type III coated charcoal (○) on the amount of <sup>57</sup>Co-B<sub>12</sub> bound by human gastric juice.

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 B<sub>12</sub> values of greater than 90%. In order to relate these results to the pH treatment of the coated charcoal, we expressed the bound <sup>57</sup>Co-B<sub>12</sub> in each tube as a percentage of the highest value of bound <sup>57</sup>Co-B<sub>12</sub> 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 <sup>57</sup>Co-B<sub>12</sub> 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 B<sub>12</sub> from bound vitamin B<sub>12</sub>. 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 B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> by the salivary vitamin B<sub>12</sub> binder (Shum, Bandouvakis, Newman, and O'Neill, 1972).

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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.

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