# Intrinsic Factor-Mediated Binding of Cyanocobalamin to Cholestyramine

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**Abstract** □ The intrinsic factor-mediated binding of cyanocobalamin to cholestyramine was studied *in vitro* under varying conditions of pH, added electrolyte, and bile salt. The intrinsic factor–cyanocobalamin complex was adsorbed strongly by the resin at pH 3 in the presence of neutral salt and low concentrations of glycocholic acid. An increase in glycocholic acid concentration as well as neutral pH decreased the observed binding, although significant amounts of cyanocobalamin–intrinsic factor complex remained bound after incubation at pH 6.8. Cyanocobalamin alone was not adsorbed by the resin.

**Keyphrases** □ Cyanocobalamin—intrinsic factor-mediated binding to cholestyramine *in vitro*, effect of pH, added electrolyte, and bile salt □ Binding, intrinsic factor mediated—cyanocobalamin to cholestyramine *in vitro*, effect of pH, added electrolyte, and bile salt □ Cholestyramine—intrinsic factor-mediated binding of cyanocobalamin, effect of pH, added electrolyte, and bile salt □ Vitamins—cyanocobalamin, intrinsic factor-mediated binding to cholestyramine *in vitro*, effect of pH, added electrolyte, and bile salt □ Ion-exchange resins—cholestyramine, intrinsic factor-mediated binding of cyanocobalamin, effect of pH, added electrolyte, and bile salt

Cholestyramine resin is effective in removing bile salts from the intestinal lumen (1, 2) and in decreasing the intragastric bile salt content (3–5). Cholestyramine also binds other compounds in the intestine, including chlorothiazide, phenylbutazone, anticoagulants, and various digitalis preparations (6). Since cholestyramine impairs the intestinal absorption of cyanocobalamin in normal patients and patients with pernicious anemia (7), it is feasible that cholestyramine decreases cyanocobalamin absorption either by binding to the sites on the intrinsic factor molecules that normally bind cyanocobalamin (7) or by binding the whole cyanocobalamin–intrinsic factor complex.

The purpose of this study was to investigate *in vitro* the molecular events in the interaction of cholestyramine with cyanocobalamin (I) and intrinsic factor as well as the effect of the bile salt, glycocholic acid, on this interaction.

## EXPERIMENTAL

**Materials**—Commercially available $^{57}$Co-labeled I[1] (10–20 μCi/μg), glycocholic acid (sodium salt)[2], and and cholestyramine[3] were used.

Normal human gastric juice was collected after stimulation with pentagastrin, depepsinized (8), diluted 1:1 with isotonic saline, and stored in aliquots at −20°. All other reagents were the highest purity commercially available.

**Incubation**—Cholestyramine particles (22.2 mg of cholestyramine) were weighed into the incubation tubes. Buffer to give a final volume of 5.25 ml was then added, followed by 300 μl of human gastric juice (12 intrinsic factor units) and 50 μl of $^{57}$Co-I (5 ng). Glycocholic acid was added, and incubation was performed at 25° by end-over-end incubation for 2 hr.

The cholestyramine particles were then collected by centrifugation, and the particles were washed in the incubation buffer and resedimented twice. The radioactivity in the final sediment was determined in an automatic β–γ-spectrometer to a precision of ±2%.

In some experiments, a buffer of a different pH was added to the sediment. The sediment was washed once and incubated in this buffer without glycocholic acid for 30 min at 25° as described. Finally, the sediment was washed twice in the buffer, and the radioactivity was determined.

Buffers of different ionic strength (0.05 and 0.1 *M* NaCl) and different pH (3.0, 6.8, and 7.4) were used in the incubations. The results were corrected by performing blank incubations, *i.e.*, incubations without cholestyramine.

## RESULTS AND DISCUSSION

The effect of pH and ionic strength on the intrinsic factor-mediated binding of $^{57}$Co-I to cholestyramine is shown in Fig. 1. A high proportion of the I–intrinsic factor complex adsorbed to the resin at pH 3 and low ionic strength. An increase in ionic strength as well as pH decreased binding. There was no significant binding of I by the resin when human gastric juice was omitted from the incubation mixture.

These results indicate that cholestyramine binds the whole I–intrinsic factor complex, which apparently contrasts with the suggestion that cholestyramine binds to the sites on the intrinsic factor molecules that normally bind I (7). However, the present incubations were done at an acid pH to simulate the pH of the stomach while the previous incubations were performed at a neutral pH (7). The observed binding of the I–intrinsic factor complex to cholestyramine at pH 3 is probably of a hydrophobic nature (9) since cholestyramine is an anion-exchange resin and the isoelectric point of the intrinsic factor has been reported to vary between 4.84 and 5.73, depending on the sialic acid content (10). Furthermore, the binding must be weak since there is competition for the exchange sites by chloride ions at low as well as neutral pH (Fig. 1).

The effect of glycocholic acid on the intrinsic factor-mediated binding

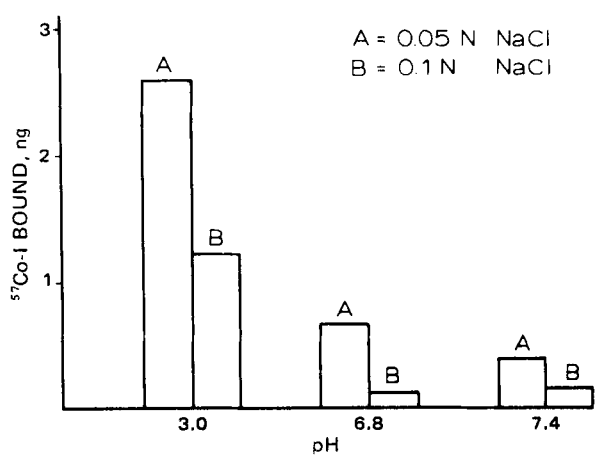


**Figure 1**—*Effect of pH and ionic strength on the intrinsic factor-mediated binding of $^{57}$Co-I to cholestyramine.*

[1] Radiochemical Center, Amersham, Bucks., England.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Questran, containing 4 g of cholestyramine chloride/9 g.



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**Table I—Effect of Glycocholic Acid, pH, and Ionic Strength on the Intrinsic Factor-Mediated Binding of I to Cholestyramine [a]**

| Glycocholic Acid Added, μmoles | Cyanocobalamin Bound, pg | | | |
|---|---|---|---|---|
| | Incubated in Buffer A | | Incubated in Buffer B | |
| | Washed in Buffer A | Incubated and Washed in Buffer C | Washed in Buffer B | Incubated and Washed in Buffer C |
| 0 | 2560 | 1370 | 1230 | 280 |
| 1.25 | 2589 | 1386 | 1247 | 262 |
| 2.5 | 3393 | 1696 | 1519 | 487 |
| 3.75 | 3465 | 2113 | 1480 | 674 |
| 5.0 | 3364 | 2444 | 605 | 257 |
| 7.5 | 3210 | 1806 | 76 | 60 |
| 10.0 | 1638 | 686 | 54 | 46 |

[a] The values given are the arithmetic means of two determinations, each run in triplicate. Incubations were performed as described in the text. The following buffers were used: 0.05 *M* NaCl, pH 3.0 (Buffer A); 0.1 *M* NaCl, pH 3.0 (Buffer B); and 0.05 *M* NaCl, pH 6.8 (Buffer C).

of I to cholestyramine at two different ionic strengths is shown in Table I. At very low concentrations of glycocholic acid, binding of the I–intrinsic factor complex to the resin increased, but when the glycocholic acid concentration was increased, complex binding gradually decreased, which suggests that glycocholic acid blocked the possible binding sites of the I–intrinsic factor complex. A further decrease in the amount of the I–intrinsic factor complex bound was observed when the ionic strength was increased by increasing the sodium chloride concentration from 0.05 to 0.1 *M* (Table I), which is compatible with the hypothesis that binding at this pH must be due to hydrophobic interactions. However, significant amounts of the complex remained bound to the resin after incubation at pH 6.8 (Table I), which suggests that the forces taking part in the binding also are coulombic.

The results show that binding of the I–intrinsic factor complex was partly reversible at neutral pH as well as at a glycocholic acid concentration similar to that found in the lumen of the small intestine. Hence, it is uncertain whether cholestyramine binds sufficient quantities of the complex in the lumen of the small intestine to impair intestinal vitamin absorption. Furthermore, results of absorption tests are conflicting; *i.e.*, cholestyramine increased as well as decreased intestinal vitamin absorption (11).

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# Simultaneous Programmed Temperature GLC Assay of Phenol, Chloroxylenol, and Lidocaine Hydrochloride in Topical Antiseptic Cream


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**Abstract** □ A simultaneous programmed temperature GLC assay for the active ingredients in a topical antiseptic cream is described. The sample is extracted directly using a dimethyl sulfoxide solution of *p*-cresol, 4-chlorophenol, and 2-amino-4-phenylthiazole as internal standards for phenol, chloroxylenol, and lidocaine hydrochloride, respectively. The resulting solution is chromatographed by temperature programming on an OV-225 column from 90 to 225°. The internal standard calculation is accomplished using peak heights or peak areas. The relative standard deviation of all assays is less than 2%.



**Keyphrases** □ Phenol—GLC analysis simultaneously with other active ingredients in commercial preparations □ Chloroxylenol—GLC analysis simultaneously with other active ingredients in commercial preparations □ Lidocaine hydrochloride—GLC analysis simultaneously with other active ingredients in commercial preparations □ GLC—simultaneous analyses, phenol, chloroxylenol, and lidocaine hydrochloride in commercial preparations


Existing assay methods for products with multiple active ingredients can often be tedious and time consuming. Current procedures for phenol, chloroxylenol (*p*-chloro-*m*-xylenol), and lidocaine hydrochloride in a topical cream formulation required 2 days of laboratory time and three separate assays for the active ingredients. A simultaneous procedure for the three active ingredients was desirable. In addition, a specific method was necessary for stability-indicating purposes.

GLC has been used successfully to determine lidocaine (1–3), phenol (4–6), and chloroxylenol (7–9). These techniques have inherent specificity qualities. The range of boiling points and polarities of these three components required a programmed temperature method.

## EXPERIMENTAL

**Materials and Reagents**—Phenol and lidocaine hydrochloride meeting USP specifications were used as standards. Chloroxylenol standard material was assayed by a GLC procedure. *p*-Cresol, 4-chlorophenol, and 2-amino-4-phenylthiazole at 99+ % purity were used as received[1]. All other chemicals were ACS reagent grade or equivalent.

The column was 1.8-m × 3-mm silanized stainless steel filled with 3% OV-225 on 80–100-mesh Supelcoport adapted for on-column injection.

[1] Aldrich Chemical Co. and Fairfield Chemical Co.


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