The Interaction Between Cholestyramine and Drugs. (30443)

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Cholestyramine is an insoluble, quarternary ammonium anion exchange resin which when administered orally to man(1,2) and animals (3,4) binds bile acids and increases the fecal elimination of bile acids and of neutral sterols. The increased excretion of the latter presumably results from the diminished concentration of free bile acids in the intestine, these being necessary for the absorption of neutral sterols(5). The effect of cholestyramine on sterol and bile acid excretion accounts for its usefulness in treatment of hypercholesteremia(3,4,6) and of the pruritis associated with biliary cirrhosis(1,2,7).

When given to patients receiving other drugs as well, cholestyramine could conceivably bind these drugs and decrease their absorption from the intestine. Therefore, a study was made of the possible interaction between the resin and several therapeutic agents which are administered orally. These studies were conducted *in vitro* as well as *in vivo,* and included examples of anionic, cationic and neutral drugs of those most commonly used.

Materials and methods. In vitro *studies.* The cholestyramine resin used contained approximately 10% moisture and was ground to pass through a 200-mesh sieve. The cationic and neutral drugs were prepared in 0.15 M phosphate buffers adjusted to pH levels of 2.0, 5.0, or 7.5. The concentration of drug used was calculated from the usual dose in humans on a weight basis so that the ratio between the drug and resin would approximate that likely to be encountered in clinical practice. The anionic drugs were also studied in this manner. In addition, the latter were equilibrated with the resin at a constant ratio of 1:100, respectively, based on their equivalent weights. For this purpose the equivalent weight of cholestyramine was assumed to be 230. Ten ml aliquots of the drug solutions and 100 mg portions of the resin were placed in 50 ml plastic centrifuge tubes and the mixture shaken at a rate of 180 oscillations per minute for 15 minutes at room temperature. Preliminary studies indicated that equilibrium between resin and drug would be completed under these conditions. Following equilibrium the tubes were centrifuged at 6000 $\times$ *g* for 5 minutes and 7.0 ml of the supernatant withdrawn for analysis, with care taken to avoid disturbing the finely divided resin deposit.

The reversibility of binding was determined by washing the drug-resin complex 4 times with fresh buffer. The complex was washed by equilibrating it with buffer for 5 minutes; additional quantities of drug could not be removed by incubating the complex with buffer for a longer period. The concentration of drug in the supernatant was generally determined by optical density measurements using a Beckman Model DU spectrophotometer at a wavelength of maximum absorbance for the drug. Digoxin was determined colorimetrically(8).

Sodium cholate-carboxyl-C^{14}, acetylsalicylic-carboxyl-C^{14} acid, phenobarbital-2-C^{14} and nicotinic-7-C^{14} acid were obtained from New England Nuclear Corp. and found by paper chromatography to be essentially radiochemically pure. Determination of C^{14} was made by adding one ml of sample to 15 ml of a counting solution—consisting of 100 g naphthalene, 7 g of 2,5-diphenyloxazole, 0.5 g of 1,4-*bis*-2-(4-methyl-5-phenyl-oxazolyl) benzene and 40 g Cab-O-Sil (thickening agent) per liter of dioxane—and measuring the radioactivity under "optimal window" conditions for this solvent system in a Tri-Carb® scintillation spectrometer.

In vivo *studies.* The effect of cholestyramine on the intestinal absorption of drugs was generally determined by administering these substances together to groups of 10 rats and measuring subsequent changes in plasma drug levels. For the studies on chlorothiazide a group of 6 beagle dogs was used; in this case, in addition to the plasma levels, the 24-hour urinary excretion of the drug was also meas-

ured. In the experiments in which warfarin was administered to rats, prothrombin times, as well as plasma drug levels, were determined.

The animals were fasted overnight and given by stomach tube a solution or suspension of the drug in 0.5% methocel; the resin, when given, was administered mixed with the drug, or in the case of chlorothiazide, one-half hour after the drug. The doses of cholestyramine resin used, 71.5 and 357.5 mg/kg, correspond on a weight basis to 5 and 25 g doses in a 70-kg person. Drugs were given on a body weight basis, the dose generally being equivalent to the usual dose in man. However, larger quantities of tetracycline and warfarin were administered to obtain measurable plasma levels.

Rats were bled by an orbital bleeding technique; with dogs, blood was obtained from the jugular vein. The plasma concentration of drug containing C^{14} was determined by suspending plasma aliquots in counting solution and measuring the radioactivity as described above. Non-radioactive drugs were determined as follows: tetracycline, fluorometrically as described by Kohn(9); phenylbutazone by the method of Burns *et al*(10); chlorothiazide as described by Baer *et al* (11); and warfarin according to O'Reilly (12). Prothrombin times were determined at plasma dilutions of 1/12, 1/8 or 1/4 by the procedure described in the Warner-Chilcott Manual of Blood Coagulation Techniques, 2nd Edition. A Mechrolab Clot Timer, Model 202, was used to measure actual clotting times. Statistical analyses of the results were performed using the "t" test of significance (13).

Results and discussion. In vitro *studies. Cationic and neutral drugs.* Cationic drugs are principally amines and amine derivatives. While a specific reaction would not be expected between cholestyramine, an anion exchange resin, and cationic drugs they could be adsorbed by the resin in a non-specific manner. The results obtained with 4 members of this class—dextromethorphan, dihydrocodeinone bitartrate, chlorpheniramine maleate and quinidine sulfate—indicated that only the latter was removed from solution by the resin to an appreciable extent (26% at pH 7.5), and subsequent experiments showed that this drug was readily washed from the resin.

TABLE I. Relative Affinity of Cholestyramine for Various Drugs at a Constant Resin:Drug Ratio.

Drug	Binding	
	Initial*	Washed†
Sodium cholate	83%	55%
Warfarin	97	95
Phenylbutazone	98	94
Chlorothiazide	88	70
Hydrochlorothiazide	86	58
Phenobarbital	58	18
Aspirin	32	8
Tetracycline	60	0
Quinidine	28	4

* 100 mg (434 μequivalents) of cholestyramine were equilibrated with 10 ml of 0.15M phosphate buffer, pH 7.5, containing 4.34 μequivalents of drug.

† Washed 4 times with 0.15M phosphate buffer after initial incubation of drug and resin.

Neutral or non-ionic compounds also should not interact with cholestyramine by an ion-exchange mechanism, although they may be adsorbed by the resin. Few substances of pharmacological interest are non-ionic in character and only digoxin was studied. The resin removed 33% of the digoxin from solution. Furthermore, as expected with a non-ionizable material, the pH of the suspending medium did not influence the amount of drug adsorbed. The weak nature of the interaction between cholestyramine and digoxin was shown by the recovery of the adsorbed drug after washing the complex with fresh buffer.

Anionic drugs. The results of the *in vitro* studies with anionic drugs are presented in Table I. Sodium cholate, a representative bile acid, was studied for comparative purposes. Eighty-three per cent of the cholate in solution was initially bound by the resin. Following 4 washes of the resin-cholate complex, 55% remained associated with the resin.

Warfarin and phenylbutazone were bound more strongly by the resin than was cholate. In the case of warfarin, 97% was initially bound, and after washing the resin 4 times with fresh buffer, 95% remained bound. Phenylbutazone behaved similarly. Chlorthiazide and hydrochlorothiazide were bound

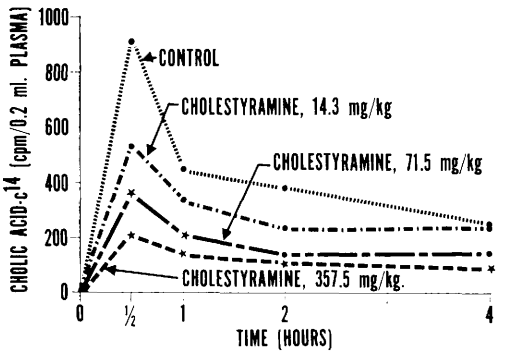

FIG. 1. Effect of cholestyramine on plasma levels of orally administered cholic acid. Suspensions of indicated amounts of cholestyramine in a solution of cholic acid-carboxyl-C^{14}, sodium salt, were given by stomach tube to fasted rats. Points indicated by stars were statistically different from control values ($P<0.05$).

approximately the same extent as cholate, while other anionic drugs such as phenobarbital, aspirin, and tetracycline were bound to a lesser extent. Quinidine, which ionizes to give a cation, was adsorbed somewhat more strongly than other amine drugs but was readily recovered by washing. Thus, the extent and stability of the complex formed between the resin and anionic drugs is not related to acidity of the drug. Aspirin (pKa 3.5) is a stronger acid than is cholate (pKa 6.4), but was not bound as tightly by the resin. Similarly, warfarin, which has a functional enol group, is a weaker acid than cholate, but was bound more strongly by the resin.

In vivo *studies.* Since a variety of conditions influence the binding and elution of drugs in the gastrointestinal tract, the effect of cholestyramine on intestinal absorption of the anionic drugs listed in Table I was determined by administering the drugs orally, with or without resin, to groups of rats or dogs and measuring subsequent changes in plasma drug levels.

Sodium cholate. The effect of cholestyramine on plasma cholic acid levels of rats given an oral dose of 11.1 mg cholic acid-C^{14} (40 μcuries) per kg is presented in Fig. 1. In animals receiving one-fifth the "normal" dose of cholestyramine (14.3 mg per kg), plasma cholic acid levels were 26 to 42% lower (not statistically significant) than in the control animals during the first 2 hours. When an approximately "normal" dose of cholestyramine (71.5 mg/kg) was given, the plasma cholic acid levels were reduced 61% the first one-half hour, and were still depressed 44% after 4 hours; however, only during the first hour were the differences statistically significant. With the high dose of cholestyramine (357.5 mg/kg), plasma cholic acid levels were decreased more than 60%, which was statistically significant, throughout the 4-hour period of the experiment.

Tetracycline. Tetracycline levels in the plasma of rats during the 4-hour period following oral administration of the antibiotic, with and without the resin, are presented in Fig. 2-A; the dose of antibiotic given was 71.4 mg/kg body weight. No statistically significant differences were observed between control and resin-treated groups, indicating that tetracycline bound by the resin was readily removed in the intestine.

Nicotinic acid. The results of a similar study in which nicotinic acid-7-C^{14} was administered at a dose of 15.9 mg/kg are presented in Fig. 2-B. The results showed that in contrast to tetracycline, the absorption of nicotinic acid by rats appeared to be slightly retarded by the larger dose of resin, but remained unaffected by the smaller dose. However, none of the differences were statistically significant.

Aspirin. Acetylsalicylic carboxyl-C^{14} acid was administered orally at a dose of 4.65 mg per kg. The results in Fig. 2-C show that the "normal" dose of cholestyramine resin did not appreciably alter the appearance of the C^{14} label in the plasma, although the larger dose of resin significantly reduced aspirin absorption throughout the 2-hour study ($P<0.05$).

Phenobarbital. Phenobarbital-2-C^{14} was given at a dose of 1.2 mg/kg. Its absorption was retarded by the lower dose of resin, as indicated by significantly lower plasma levels at the 15- and 30-minute time intervals. However, no differences from the control were observed at later time intervals (Fig. 2-D). As with aspirin, the larger dose of resin significantly reduced absorption at all time intervals studied ($P<0.05$).

Chlorothiazide. The effect of cholestyra-

mine resin on chlorothiazide absorption was studied in dogs. Six male beagle dogs, ranging in weight from 8 to 13 kg, were fasted overnight and given by gavage a suspension of chlorothiazide in 0.5% methocel at a dose of 14.3 mg/kg. Thirty minutes later each dog was given 100 g of a meat based dog food, with half the dogs receiving cholestyramine mixed with the food at a dose of 71.5 mg/kg body weight. The food was consumed in about 2 minutes. Plasma chlorothiazide levels were determined 30, 60 and 120 minutes after chlorothiazide administration and total urinary excretion of chlorothiazide was determined on 24-hour urine collections.

This experiment was repeated at weekly intervals until each dog had been studied twice after receiving the resin with food and drug and twice after receiving only food and drug. The two values obtained for each dog for each treatment were averaged and considered as a single value for statistical analysis.

The results of this experiment are presented in Fig. 3. The administration of cholestyramine reduced plasma chlorothiazide levels 22% 30 minutes after the resin was given, and 52% 90 minutes after treatment with resin. These differences were not statistically significant ($P>0.05$).

Chlorothiazide is not metabolized by the dog, and its urinary excretion occurs rapidly and is dose related(11). Thus, the urinary excretion of chlorothiazide can be used as a measure of its absorption. In the present experiment, dogs in the control group excreted 48% of the dose in the 24-hour period following its administration, while the resin-treated

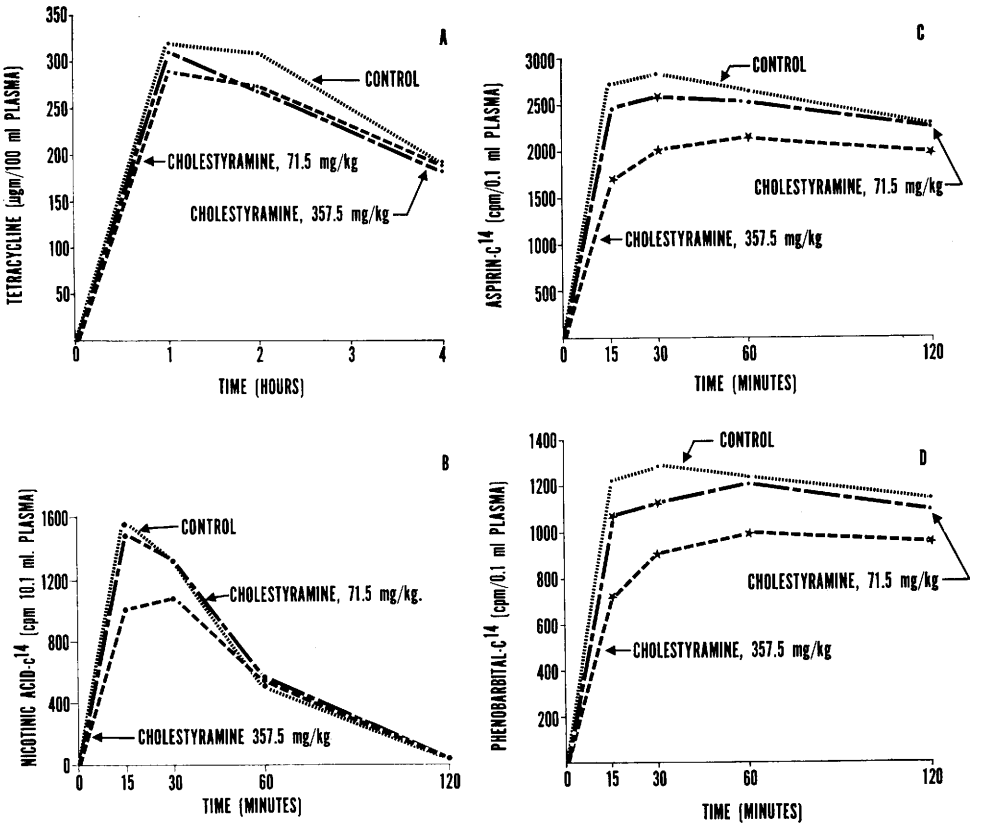

FIG. 2. Effect of cholestyramine on intestinal absorption of selected anionic drugs. Resin was given by stomach tube to fasted rats as a suspension in the drug solution. (A) Tetracycline, (B) nicotinic-7-C^{14} acid, (C) acetylsalicylic-carboxyl-C^{14} acid, (D) 5-ethyl-5-phenylbarbituric-2-C^{14} acid. Stars indicate statistically significant differences from control values ($P<0.05$).

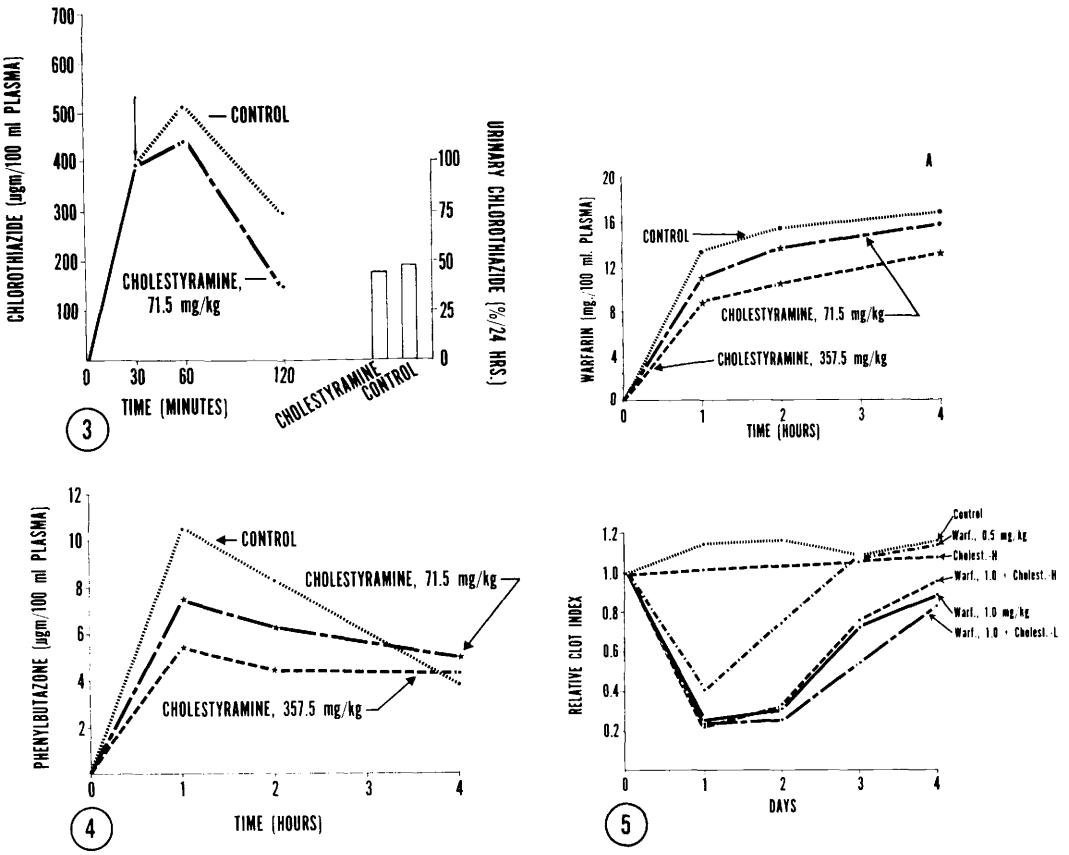

FIG. 3. Influence of cholestyramine on chlorothiazide absorption in dogs. Cholestyramine was administered with food one-half hour (arrow) after an oral dose of chlorothiazide.

FIG. 4. Effect of cholestyramine on plasma levels of orally administered phenylbutazone. Stars indicate statistically significant differences from control values ($P<0.05$).

FIG. 5. (A) Effect of cholestyramine on intestinal absorption of warfarin. Stars indicate statistically significant differences from control values. (B) Changes in relative clot index of rats administered cholestyramine and warfarin. Relative clot index = initial prothrombin time/experimental prothrombin time. Cholest-H indicates a cholestyramine dose of 357.5 mg/kg; Cholest-L indicates a dose of 71.5 mg/kg.

dogs excreted 43% (Fig. 3). This difference was not statistically significant.

Phenylbutazone. The effect of the resin on the intestinal absorption of phenylbutazone was studied in fasted rats using the same general procedures described previously. The results presented in Fig. 4 show that after 1 hour, in the animals receiving the lower dose of resin (71.5 mg/kg), plasma phenylbutazone levels were reduced 32%; the higher dose (357.5 g per kg) caused a reduction of 49%. After 2 hours, reductions of 22 and 47% were observed with the low and high doses of resin, respectively. These changes were statistically significant ($P<0.01$). However, 4 hours after administration of the resin, the group receiving the lower dose of cholestyramine had a plasma level which was 29% greater than that of the control ($P<0.05$), while the level in the high-dose group was the same as that of the control. These data suggest that the resin retarded rather than diminished phenylbutazone absorption.

Warfarin. The effect of the resin on warfarin absorption was studied in rats as described above. It was necessary to use a warfarin dose of 100 mg per kg, which far exceeds the toxic dose in rats, in order to obtain measurable plasma levels.

Compared to the other drugs studied, warfarin was more slowly absorbed from the intestinal tract, as indicated by the increase in

plasma levels in all the groups throughout the 4 hours of study (Fig. 5-B). As with phenylbutazone, both doses of cholestyramine significantly lowered plasma warfarin levels 1 and 2 hours after treatment. However, unlike phenylbutazone, in the group given the higher dose of resin, plasma warfarin remained significantly depressed after 4 hours (—21%; $P<0.01$); the plasma level of the group receiving the lower dose of cholestyramine was only slightly lower (—6%) than that of the control at this time interval.

A second study was conducted to determine the effect of cholestyramine on the anticoagulant action of warfarin. A single dose of warfarin was given to rats at levels of 0.5 to 1.0 mg/kg. Additional groups received warfarin at 1.0 mg/kg simultaneously with a single dose of cholestyramine at 71.5 or 357.5 mg/kg. Other groups were given either cholestyramine alone, at a dose of 357.5 mg/kg, or a control solution. Plasma prothrombin times were determined on diluted plasma samples prior to treatment and for the following 4 days, and the relative clot index, which is the ratio of the initial prothrombin time to the post-treatment prothrombin times, was calculated.

The results of this study, presented in Fig. 5-B, show that neither the high nor low dose of cholestyramine significantly affected the relative clot index, as compared to the group which received warfarin only. Thus, although cholestyramine may delay the early absorption of warfarin, the pharmacological effect of a single administration of the drug was not significantly decreased by a single dose of resin.

The results of the present study indicate that the gastrointestinal absorption of some agents was retarded by the concomitant administration of the resin; but in those cases which were further investigated the total amount absorbed was not significantly reduced. Nevertheless, continued administration of the resin could produce an effect somewhat different from those observed in the present investigation, in which only a single dose was studied. Consequently, it would be prudent to administer drugs (orally) a short time before cholestyramine to preclude delay of drug action.

Summary. A study was made of the *in vitro* and *in vivo* interaction between cholestyramine resin and several commonly used, orally administered drugs. Basic and neutral substances were bound only weakly, if at all, *in vitro*. Also, many anionic drugs were not strongly bound by cholestyramine, and the gastrointestinal absorption of those drugs which were strongly bound was only moderately delayed by concurrent administration of a single dose of resin.

The authors wish to express their gratitude to Messrs. E. M. Medler, J. S. Bogard and J. A. Becker, and Mrs. Catherine Kohl for valuable assistance and to Dr. W. M. Cox, Jr. for helpful discussions during the course of this study.

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Received May 4, 1965. P.S.E.B.M., 1965, v120.