(4) W. A. Abruzzi, *ibid.*, **71**, 329(1964).
(5) E. Novak, C. E. Seckman, T. G. Vitti, D. J. Chodos, R. L. Hyde, and W. J. Clemens, *Int. Med. Dig.*, **5**, 37(1970).
(6) R. J. Matthews, J. P. Davanzo, R. J. Collins, and M. J. VanderBrook, *Arch. Int. Pharmacodyn. Ther.*, **143**, 574(1963).
(7) G. Slomp, R. H. Baker, and F. A. MacKellar, *Anal. Chem.*, **36**, 375(1964).
(8) A. A. Forist and R. W. Judy, *J. Pharm. Sci.*, **53**, 1244(1964).
(9) W. E. Hamlin, J. I. Northam, and J. G. Wagner, *ibid.*, **54**, 1651(1965).
(10) E. F. Salim and R. E. Booth, *ibid.*, **56**, 623(1967).
(11) T. W. M. Davis, *J. Chromatogr.*, **29**, 283(1967).
(12) A. M. Gillespie and S. M. Walters, *Anal. Lett.*, **6**, 61(1973).
(13) D. R. Buhler, *J. Pharmacol. Exp. Ther.*, **145**, 232(1964).
(14) D. R. Buhler, *Biochem. Pharmacol.*, **14**, 371(1965).
(15) D. R. Buhler, H. Harpootlian, and R. L. Johnston, *ibid.*, **15**, 1507(1966).
(16) R. C. Thomas, D. R. Buhler, and G. J. Ikeda, *J. Pharm. Sci.*, **56**, 507(1967).
(17) A. A. Forist and R. W. Judy, *ibid.*, **60**, 1686(1971).
(18) A. M. Morgan, E. B. Truitt, and J. M. Little, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 374(1957).
(19) E. Titus, S. Ulick, and A. P. Richardson, *J. Pharmacol. Exp. Ther.*, **93**, 129(1948).
(20) J. F. Douglas, J. A. Stockage, and N. B. Smith, *J. Pharm. Sci.*, **59**, 107(1970).
(21) W. R. Maynard, Jr., and R. B. Bruce, *ibid.*, **59**, 1346(1970).
(22) P. Adams and F. A. Baron, *Chem. Rev.*, **65**, 567(1965).
(23) L. Fishbein and W. L. Zielinski, Jr., *Chromatographia*, **2**, 38(1969).
(24) L. Fishbein and W. L. Zielinski, Jr., *Chromatogr. Rev.*, **9**, 37(1967).
(25) L. Fishbein and W. L. Zielinski, Jr., *J. Chromatogr.*, **20**, 9(1965).

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[x] To whom inquiries should be directed.

Effects of Colestipol Hydrochloride on Drug Absorption in the Rat I: Aspirin, L-Thyroxine, Phenobarbital, Cortisone, and Sulfadiazine

WILLIAM A. PHILLIPS[x], JOHN R. SCHULTZ, and WALTER W. STAFFORD

Abstract □ The effects of colestipol hydrochloride, a hypocholesterolemic bile acid-binding anion-exchange polymer, on the GI absorption of five drugs commonly used in humans were studied in the rat. Colestipol hydrochloride was given orally in single doses of 71.5 or 214.5 mg/kg, equivalent to 5 (usual single dose) or 15 g, respectively, in a 70-kg human; controls received equal amounts of microcrystalline cellulose. Single oral doses of labeled drugs were given concurrently with colestipol hydrochloride and control in the human therapeutic dose range on a milligram per kilogram basis. Subsequent changes in serum drug levels were measured at several time intervals and evaluated mathematically by a one-compartment open model. The high dose of colestipol hydrochloride reduced the rate of absorption of aspirin-carboxyl-^{14}C from 8.36 to 4.68 hr^{-1} and increased the absorption half-life from 0.102 to 0.165 hr. Peak serum radioactivity was reduced by 27%, and the area under the time–concentration curve was reduced by 15%. The low dose of colestipol hydrochloride reduced peak radioactivity and the area under the curve of L-thyroxine-^{14}C (uniformly labeled) by 22 and 25%, respectively. The high dose of colestipol hydrochloride also reduced these parameters; however, the reduced total absorption increased the apparent rate of absorption from 0.169 to 0.270 hr^{-1} and reduced the absorption half-life from 4.24 to 2.68 hr. Colestipol hydrochloride did not affect the absorption of phenobarbital-2-^{14}C, cortisone acetate-4-^{14}C, or sulfadiazine-^{35}S. These results indicate that colestipol hydrochloride can inhibit absorption of some concurrently administered drugs from the GI tract of the rat.

Keyphrases □ Colestipol hydrochloride—effects on aspirin, L-thyroxine, phenobarbital, cortisone, and sulfadiazine absorption, rat □ Absorption, drug—effects of colestipol hydrochloride on various drugs, rat □ Antilipemic agents—effects of colestipol hydrochloride on absorption of aspirin, L-thyroxine, phenobarbital, cortisone, and sulfadiazine, rat □ Anion-exchange polymers, colestipol hydrochloride—effects on absorption of various drugs, rat

Colestipol hydrochloride[1] is a high molecular weight anion-exchange polyethylenepolyamine polymer with 1-chloro-2,3-epoxypropane which may have approximately one of five amine nitrogens protonated as the chloride salt. Investigators have shown that orally administered polymer binds bile salt anions in the small intestine and reduces serum cholesterol levels in experimental animals (1–3) and in humans (4–8). Since colestipol may also bind other substances, including drugs that might be used as concurrent therapy in hypercholesterolemic subjects, the effects of the polymer on the absorption of a series of drugs in the rat were studied. Results with radiolabeled aspirin, L-thyroxine, phenobarbital, cortisone, and sul-

[1] Colestid, The Upjohn Co., Kalamazoo, Mich. The official generic name (USAN) for the material reported here is colestipol hydrochloride; colestipol is used as an abbreviation in the text.

Table I—Kinetic Parameters for Aspirin with Concurrent Administration of Colestipol or Cellulose Control

Parameters[a]	Dose, 71.5 mg/kg		Dose, 214.5 mg/kg		Standard Deviation, %[d]
	Control[b]	Colestipol	Control	Colestipol[c]	
Model:					
K_A[e]	6.55	6.44	8.36	4.68*	49.7
$A_{1/2}$	0.111	0.116	0.102	0.165*	51.3
K_E	0.161	0.179	0.177	0.156	24.9
$E_{1/2}$	4.65	4.08	4.37	4.67	33.0
Peak radioactivity	4,900	4,702	5,052	3,759**	13.1
Area	34,960	30,313	34,129	28,210	25.2
Observed:					
Peak radioactivity	4,860	4,628	5,012	3,641**	12.7
Area	29,005	25,882	27,804	23,671*	15.7

[a] K_A(hr^{-1}) = rate of absorption; $A_{1/2}$(hr) = absorption half-life; K_E(hr^{-1}) = rate of elimination; $E_{1/2}$(hr) = elimination half-life; peak radioactivity is in dpm/0.1 ml serum, and area under the time–concentration curve is in dpm/0.1 ml × hr. The abbreviations are the same in all tables. [b] Represents mean of nine animals; all other values represent mean of 10 animals. [c] Significant difference between colestipol means and mean of corresponding control at (*) $p < 0.05$ and (**) $p < 0.01$. [d] Obtained from analysis of variance over all four groups. [e] Data converted to logarithms to achieve more homogeneous within-group variances; values expressed as antilog of logarithm means.

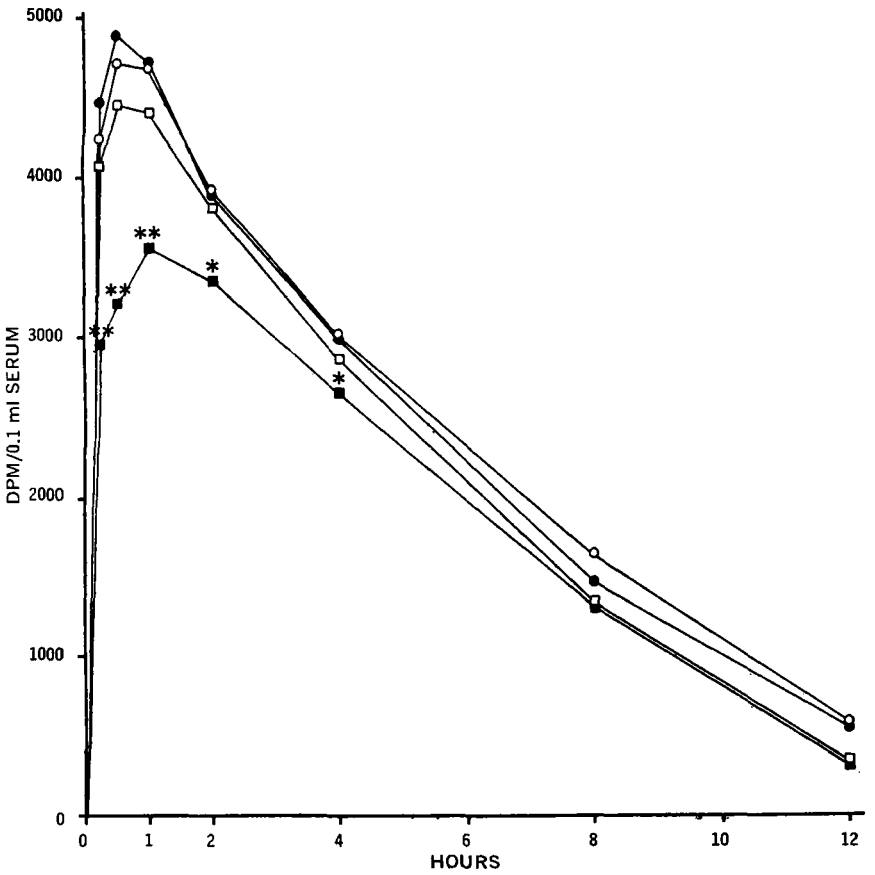


Figure 1—*Effect of colestipol on absorption of aspirin-carboxyl-^{14}C. Key: cellulose control at 71.5 (○) and 214.5 (●) mg/kg; colestipol at 71.5 (□) and 214.5 (■) mg/kg; and significant difference between colestipol mean and mean of corresponding control at* $p < 0.05$ (*) *and* $p < 0.01$ (**).

fadiazine, whose pharmacokinetics can be described by a one-compartment open model, are reported here.

EXPERIMENTAL

Ten male rats[2] with an average weight of approximately 240 g were used in each group. The rats were fasted for 18 hr prior to and during each experiment; water was allowed *ad libitum*. Colestipol was suspended in 0.25% aqueous methylcellulose and administered by stomach tube at 71.5 and 214.5 mg/kg. Controls received equal amounts of a bulk material, microcrystalline cellulose[3], suspended and dosed similarly to colestipol. These levels correspond on a weight basis to 5 and 15 g, respectively, of material in a 70-kg human. After treatment, each rat was immediately, within 10 sec, administered the radioactive drug dissolved or suspended in 0.25% methylcellulose vehicle by stomach tube. The total doses of the drugs, after addition of nonradioactive compound[4], ranged generally within the amount given to humans on a weight basis. In the sulfadiazine experiment, each rat received about 1.9 μCi/100 g body weight; in all other experiments each received about 0.45 μCi. The rats were then bled from the jugular vein (0.25 ml) at various time periods (9). Radioassay of 0.1 ml serum was made in a liquid scintillation spectrometer[5]; quenching was determined using the automatic external standard and a prepared efficiency correlation curve. Correction for ^{35}S decay was also made in the sulfadiazine experiment. The scintillation fluid

[2] Upj:TUC(SD)spf.
[3] Avicel, PH-102, FMC Corp., American Viscose Division, Marcus Hook, Pa.
[4] Aspirin, phenobarbital, cortisone acetate, and sulfadiazine were USP grade; supplementation with L-thyroxine was not necessary.
[5] Model 3375, Packard Instrument Co., Downers Grove, Ill.

Table II—Kinetic Parameters for L-Thyroxine with Concurrent Administration of Colestipol or Cellulose Control

Parameters	Dose[a], 71.5 mg/kg		Dose[a], 214.5 mg/kg		Standard Deviation, %
	Control	Colestipol[b]	Control	Colestipol[b]	
Model:					
K_A	0.176	0.221	0.169	0.270**	27.4
$A_{1/2}$	4.08	3.21	4.24	2.68**	27.9
K_E	0.028	0.028	0.023	0.027	29.7
$E_{1/2}$	26.5	27.1	32.5	28.0	29.8
Peak radioactivity	1,352	1,050**	1,476	1,081**	12.3
Area	72,650	54,150*	93,046	55,385**	25.6
Observed:					
Peak radioactivity	1,420	1,112**	1,564	1,153**	12.9
Area	58,222	43,375**	67,905	43,639**	15.6

[a] Mean values represent 10 animals per group. [b] Significant difference between colestipol mean and mean of corresponding control at (*) $p < 0.05$ and (**) $p < 0.01$.

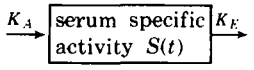
Table III—Kinetic Parameters for Phenobarbital with Concurrent Administration of Colestipol or Cellulose Control

Parameters	Dose, 71.5 mg/kg		Dose, 214.5 mg/kg		Standard Deviation, %
	Control[a]	Colestipol	Control	Colestipol	
Model:					
K_A[b]	7.63	6.84	5.69	5.22	52.3
$A_{1/2}$	0.106	0.116	0.135	0.138	47.5
K_E	0.088	0.072	0.082	0.085	20.5
$E_{1/2}$	8.45	9.92	8.62	8.32	20.3
Peak radioactivity	1,196	1,164	1,197	1,198	8.5
Area	15,219	17,427	15,801	15,364	17.1
Observed:					
Peak radioactivity	1,214	1,168	1,197	1,187	9.2
Area	13,869	15,032	14,316	14,050	12.0

[a] Represents mean of nine animals; all other values represent mean of 10 animals. [b] Data converted to logarithms; values expressed as antilog of logarithm means.

contained 8.8% serum solubilizer[6], 0.4% 2,5-diphenyloxazole, and 0.005% *p*-bis[2-(5-phenyloxazolyl)]benzene in toluene. The radioactive drugs [aspirin-carboxyl-^{14}C[7], L-thyroxine-^{14}C (uniformly labeled)[7], cortisone-4-^{14}C acetate[7], sulfadiazine-^{35}S[7], and 5-ethyl-5-phenylbarbituric-2-^{14}C acid[8]] were found to be radiochemically pure by TLC on silica gel and scanning with a radiochromatogram scanner[9].

The statistical analysis of the data for each drug was made in the following manner. The observed specific activity curves (dpm/0.1 ml serum) for each rat were analyzed by the one-compartment open model (Scheme I):



Scheme I

where the serum activity-time function is described by:

$$S(t) = D\left(\frac{K_A}{K_E - K_A}\right)(e^{-K_A t} - e^{-K_E t}) \qquad \text{(Eq. 1)}$$

Nonlinear techniques were used to estimate the parameters of this model (10). The absorption half-life, $A_{1/2}$ (hours), was obtained as ln $2/K_A$, and the elimination half-life $E_{1/2}$ (hours) was obtained as ln $2/K_E$, where K_A (hr^{-1}) and K_E (hr^{-1}) are the rates of absorption and elimination, respectively. The curve generated for each rat with the estimated parameter values in the serum activity-time function was used to obtain the area under the curve [(dpm/0.1 ml) × hr] from time zero to infinity and peak radioactivity. Peak radioactivity was also obtained from the observed curve, and the area from time zero to the end of the test was calculated with the trapezoidal rule.

For each parameter described, a one-way analysis of variance (11) was performed on the values from the four groups of animals; comparisons were made between each colestipol mean and the mean of the corresponding control. This procedure was also carried out on the specific activity values observed at each sampling period. In some analyses the data were transformed to logarithms to achieve more homogeneous within-group variances; values were then reported as the antilog of logarithm means. When more than two samples were lost during centrifugation or the rat died from the bleeding procedure, the data from that animal were not included in the analyses.

[6] Bio-Solv BBS-3, Beckman Instruments, Inc., Fullerton, Calif.
[7] Amersham/Searle.
[8] New England Nuclear.
[9] Model 885, Vanguard Instrument Corp., La Grange, Ill.

RESULTS AND DISCUSSION

Aspirin-carboxyl-^{14}C—The results obtained with aspirin administered at 4.4 mg/kg are presented in Table I and Fig. 1. The differences in serum drug levels with 71.5 mg/kg colestipol and the corresponding control were not significant at any sampling period. Estimated model parameters for absorption, elimination, peak radioactivity, and area under the time-concentration curve (zero to infinity) did not differ significantly from control. Values for observed peak radioactivity and the area under the curve (0–12 hr) were unaltered significantly. However, 214.5 mg/kg colestipol caused a significant reduction in serum drug levels at 0.25, 0.5, 1, 2, and 4 hr. The rate of absorption of aspirin decreased (from 8.36 to 4.68 hr^{-1}) and absorption half-life increased (from 0.102 to 0.165 hr^{-1}); the rate of elimination and the elimination half-life did not vary significantly from the control values. Drug availability as measured by the area under the time-concentration curve from the model and the observed area were reduced by 17 and 15%, respectively; only the latter was significantly different from control. Model and observed peak radioactivities were both reduced significantly by 27%.

Cholestyramine in rats did not reduce absorption of aspirin at 71.5 mg/kg but did at 357.5 mg/kg, as measured by ^{14}C appearance in the plasma during a 2-hr study (12). Although the level of resin was greater than used in the present studies, the results indicate that both anion-exchange drugs can bind aspirin when administered concurrently to the rat.

L-Thyroxine-^{14}C (Uniformly Labeled)—Serum levels of L-thyroxine after oral administration of 0.029 mg/kg were measured at various time intervals over 3 days. Serum drug levels significantly

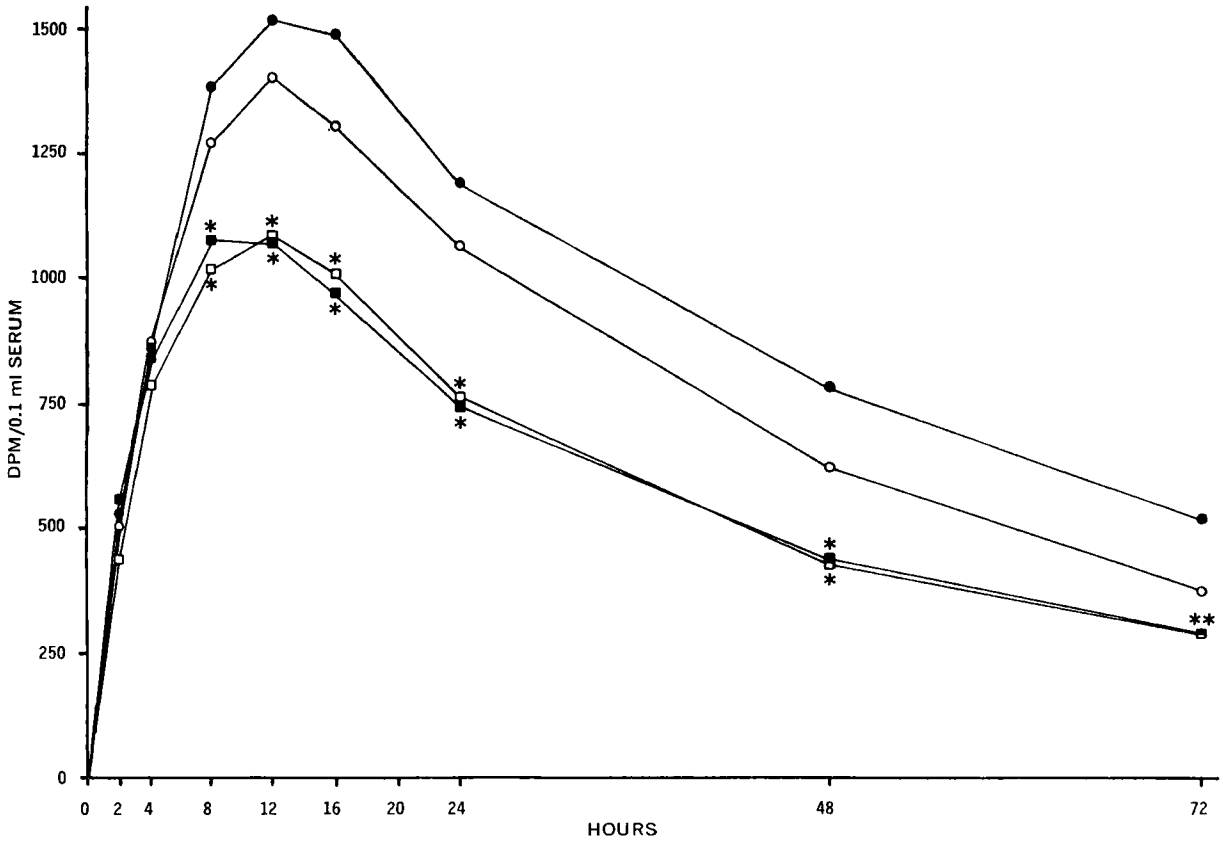


Figure 2—*Effect of colestipol on absorption of* L-*thyroxine-*^{14}C*(uniformly labeled). Key: cellulose control at 71.5* (○) *and 214.5* (●) *mg/kg; colestipol at 71.5* (□) *and 214.5* (■) *mg/kg; and significant difference between colestipol mean and mean of corresponding control at* $p < 0.01$ (*) *and* $p < 0.01$ *for high level of colestipol only* (**).

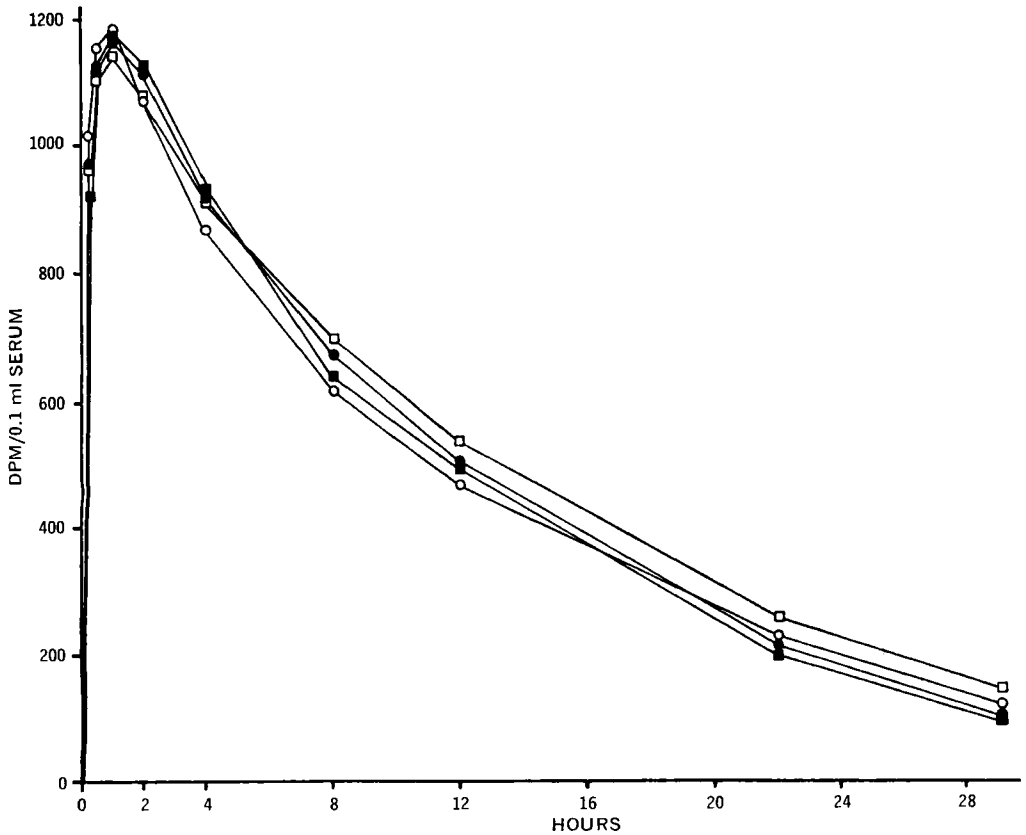


Figure 3—*Effect of colestipol on absorption of phenobarbital-2-*^{14}C*. Key: cellulose control at 71.5* (○) *and 214.5* (●) *mg/kg; and colestipol at 71.5* (□) *and 214.5* (■) *mg/kg.*

Table IV—Kinetic Parameters for Cortisone with Concurrent Administration of Colestipol or Cellulose Control

Parameters	Dose, 71.5 mg/kg		Dose, 214.5 mg/kg		Standard Deviation, %
	Control	Colestipol[a]	Control[a]	Colestipol	
Model:					
K_A[b]	9.87	12.48	7.82	9.66	46.0
$A_{1/2}$	0.079	0.059	0.092	0.082	44.0
K_E	0.939	1.037	1.122	1.124	22.0
$E_{1/2}$	0.797	0.692	0.642	0.646	25.7
Peak radioactivity	583	590	531	573	21.6
Area[b]	800	720	659	683	22.7
Observed:					
Peak radioactivity	561	581	526	571	22.0
Area	785	704	672	689	22.1

[a] Represents mean values of nine animals; all other values represent mean of 10 animals. [b] Data converted to logarithms; values expressed as antilog of logarithm means.

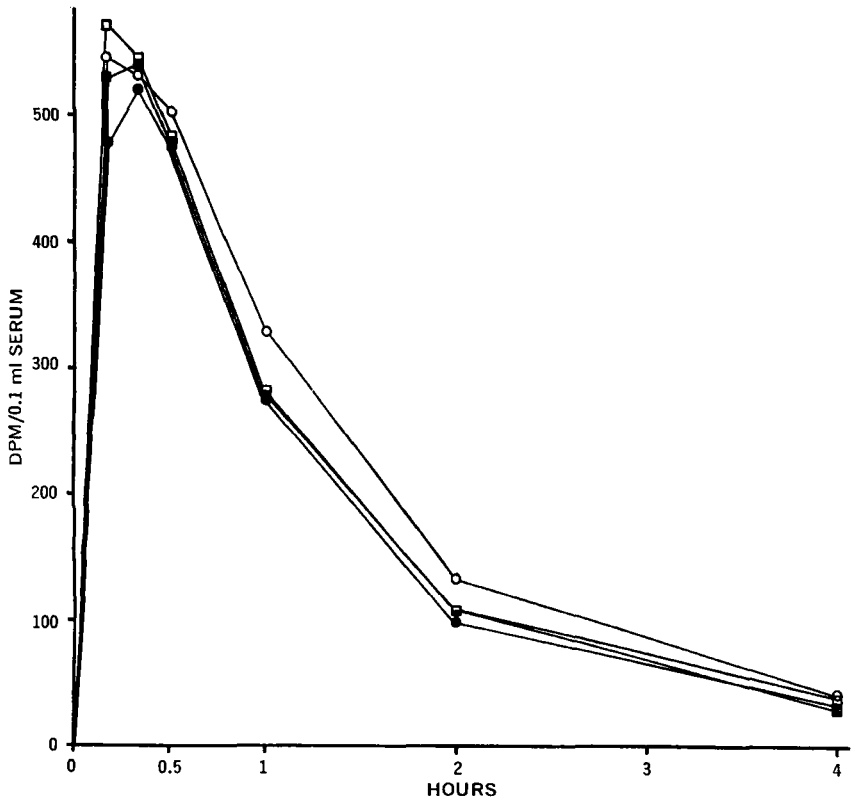


Figure 4—*Effect of colestipol on absorption of cortisone-4-^{14}C acetate. Key: cellulose control at 71.5 (○) and 214.5 (●) mg/kg; and colestipol at 71.5 (□) and 214.5 (■) mg/kg.*

decreased with the low level of colestipol at 8, 12, 16, 24, and 48 hr (Table II and Fig. 2). Model and observed peak radioactivities and areas under the curves were reduced significantly and to the same extent, 22 and 25%, respectively; none of the other changes differed significantly from control values. At the high level of colestipol, the amount of radioactivity in the serum decreased significantly at all time intervals except at the 2- and 4-hr sampling periods. There was a significant increase in the rate of absorption (from 0.169 to 0.270 hr^{-1}) and a decrease in the absorption half-life of the drug (from 4.24 to 2.68 hr). This apparent increase in the rate of L-thyroxine absorption may be due to reduced availability of the drug at the absorption site since it has been shown that overestimation of the absorption rate constant increases as bioavailability decreases (14). The rate of elimination and the elimination half-life did not vary significantly from the control values. Observed peak radioactivity and drug availability decreased significantly by 27 and 36%, respectively. Significant decreases of model values for these parameters were essentially the same as observed values.

Phenobarbital-2-^{14}C—The results obtained with 1.34 mg/kg phenobarbital-2-^{14}C appear in Table III and Fig. 3. The changes in dpm/0.1 ml serum and kinetic and observed parameters with colestipol did not differ significantly from corresponding controls.

Cholestyramine retarded the absorption of phenobarbital in the rat (12). At the lower dose of resin (71.5 mg/kg), drug plasma levels were reduced at the 15- and 30-min sampling periods; at the higher dose (357.5 mg/kg), absorption was significantly reduced at all time intervals during the 2-hr test. There appears to be a difference between the binding capacities of cholestyramine and colestipol for phenobarbital; however, comparisons should be made under identical experimental conditions.

Cortisone-4-^{14}C Acetate—Cortisone-4-^{14}C acetate was administered at 1.13 mg/kg to each rat. None of the serum radioactivities at various sampling periods or kinetic parameters was influenced at either level of colestipol (Table IV and Fig. 4).

Sulfadiazine-^{35}S—The results obtained with 14 mg/kg sulfadiazine-^{35}S appear in Table V and Fig. 5. The differences in serum radioactivities for the 71.5-mg/kg dose and the control were not significant at any sampling period. Colestipol at 214.5 mg/kg decreased radioactivity at 1 hr; other differences did not vary significantly. The changes in kinetic parameters were not significant at either level with the polymer.

Colestipol is an anion-exchange polymer that would be expected to bind with ionized acidic drugs by electrostatic forces (*e.g.*, between the negatively charged carboxyl groups of aspirin and the positively charged nitrogen atoms of colestipol). Since colestipol interfered with absorption of aspirin (pKa 3.5) but not phenobarbital (pKa 7.2), one could speculate that the stability of the

Table V—Kinetic Parameters for Sulfadiazine with Concurrent Administration of Colestipol or Cellulose Control

Parameters	Dose, 71.5 mg/kg[a]		Dose, 214.5 mg/kg[a]		Standard Deviation, %
	Control	Colestipol	Control	Colestipol	
Model:					
K_A[b]	0.30	0.32	0.32	0.31	49.8
$A_{1/2}$	2.57	2.30	2.43	2.63	51.5
K_E[b]	0.10	0.10	0.10	0.09	33.0
$E_{1/2}$	7.07	6.74	7.49	8.88	48.6
Peak radioactivity	21,657	19,977	20,620	18,411	16.6
Area[b]	386,872	337,995	408,478	408,478	27.1
Observed:					
Peak radioactivity	22,491	20,008	21,326	19,467	18.2
Area	348,726	308,770	334,263	317,547	16.2

[a] Mean values represent 10 animals per group. [b] Data converted to logarithms; values expressed as antilog of logarithm means.

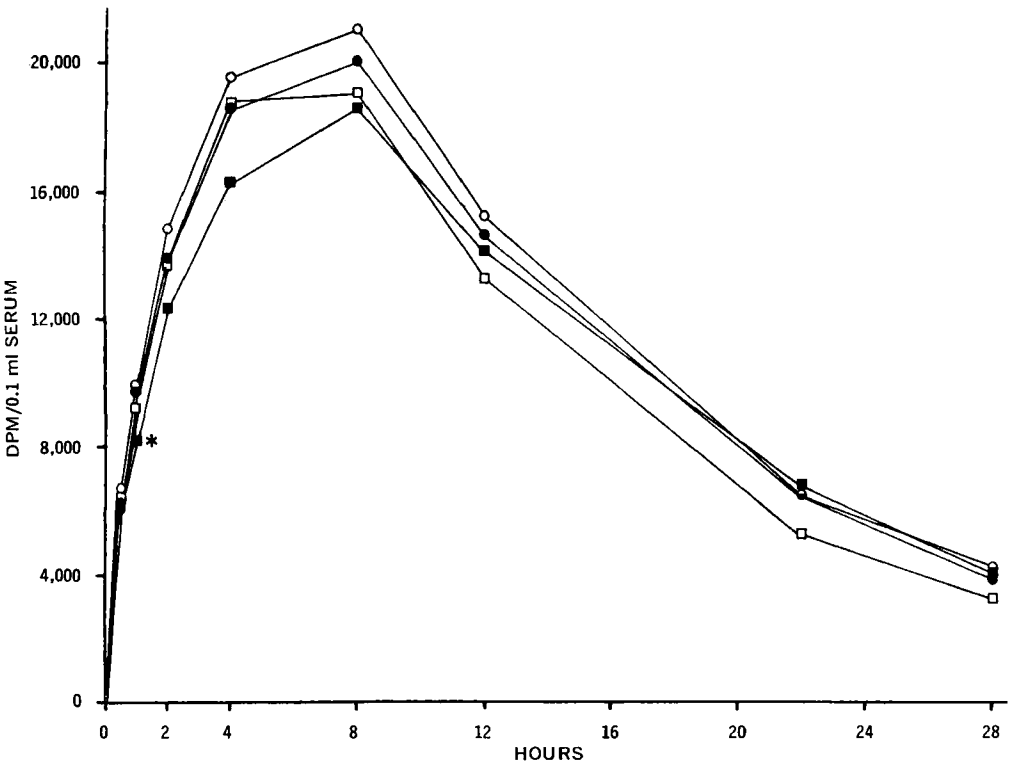


Figure 5—*Effect of colestipol on absorption of sulfadiazine-^{35}S. Key: cellulose control at 71.5 (○) and 214.5 (●) mg/kg; colestipol at 71.5 (□) and 214.5 (■) mg/kg; and significant difference between high colestipol mean and mean of corresponding control at* p *<0.05* (*).

drug–polymer interaction was related to the pKa of these drugs. This pKa relationship to drug and polymer interaction may not always determine the extent of binding, since other forces such as hydrogen bonding, dipole–dipole interactions, van der Waals forces, nonelectrostatic interactions, and intermolecular attraction of like molecules also can play an important part in the process.

The absorption of L-thyroxine was most markedly reduced by colestipol in comparison to the other drugs tested. In addition to the primary ionic interaction between the anionic carboxyl group of L-thyroxine and the basic insoluble polymer, there can be a reinforcement by a nonelectrostatic force existing between the hydrophobic portion of L-thyroxine and the polymer molecules. Evidence for the importance of the hydrophobicity of the adsorbate molecule was reported in studies with cholestyramine (14). Since the phenolic hydroxyl of L-thyroxine has a pKa of 6.73 and is over 50% ionized at physiological pH (15), it is possible that the phenolic hydroxyl may have influenced binding to the polymer by hydrogen bonding or electrostatic attraction. Although thyroxine is known to undergo marked enterohepatic circulation in the rat (16), the polymer had no significant effect on rates of excretion of drug from the serum at either level.

Colestipol had little or no effect on the absorption of the weakly acidic drugs phenobarbital (pKa 7.2) and sulfadiazine (pKa 6.5). Competition for binding sites on the polymer of these drugs with bile acid anions and inorganic physiological anions (*e.g.*, phosphate, chloride, and bicarbonate) present in the GI tract could possibly explain these results. Since the rat does not possess a gallbladder, there could be a continuous flow of bile salts and electrolytes competing for binding sites on the polymer.

The present study indicates that colestipol, like cholestyramine, can inhibit the absorption of other therapeutic agents from the GI tract of the rat. Whether results obtained in rats are predictive of results in humans remains to be determined. However, potential drug interaction could be minimized by separating administration of colestipol and other drugs as much as is practical.

REFERENCES

(1) T. M. Parkinson, K. Gundersen, and N. A. Nelson, *Atherosclerosis*, **11**, 531(1970).

(2) T. M. Parkinson, J. C. Schneider, Jr., and W. A. Phillips, *ibid.*, **17**, 167(1973).

(3) J. R. Ryan and A. Jain, *J. Clin. Pharmacol.*, **12**, 268(1972).

(4) E. Marmo, A. P. Caputi, S. Cataldi, and A. Amelio, *G. Arterioscler.*, **8**, 229(1970).

(5) C. J. Glueck, S. Ford, Jr., D. Scheel, and P. Steiner, *J. Amer. Med. Ass.*, **222**, 676(1972).

(6) E. R. Nye, D. Jackson, and J. D. Hunter, *N. Z. Med. J.*, **76**, 12(1972).

(7) D. S. Goodman, R. P. Noble, and R. B. Dell, *J. Clin. Invest.*, **52**, 2646(1973).

(8) K. Gundersen, in "Pharmacological Control of Lipid Metabolism," W. L. Holmes, R. Paoletti, and D. Kritchevsky, Eds.,

Plenum, New York, N.Y., 1972, p. 298.
(9) W. A. Phillips, W. W. Stafford, and J. Stuut, Jr., *Proc. Soc. Exp. Biol. Med.*, **143**, 733(1973).
(10) C. M. Metzler, "Compilation of Symposia Papers," 5th National Meeting, APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1970, p. 380.
(11) G. W. Snedecor and W. G. Cochran, "Statistical Methods," 6th ed., Iowa State University Press, Ames, Iowa, 1967, p. 258.
(12) D. G. Gallo, K. R. Bailey, and A. L. Sheffner, *Proc. Soc. Exp. Biol. Med.*, **120**, 60(1965).
(13) D. Perrier and M. Gibaldi, *J. Pharm. Sci.*, **62**, 225(1973).
(14) W. H. Johns and T. R. Bates, *ibid.*, **59**, 329(1970).
(15) C. L. Gemmill, *Arch. Biochem. Biophys.*, **54**, 359(1955).
(16) A. Albert and F. R. Keating, *Endocrinology*, **51**, 427(1952).

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[x] To whom inquiries should be directed.

Succinylsulfathiazole Crystal Forms I: Preparation, Characterization, and Interconversion of Different Crystal Forms

M. A. MOUSTAFA, SAID A. KHALIL[x], A. R. EBIAN, and M. M. MOTAWI†

Abstract □ Some aqueous suspensions of succinylsulfathiazole exhibited physical instability which was manifested by crystal growth, caking, formation of cement-like precipitates, and difficult resuspendability. Therefore, the polymorphism of succinylsulfathiazole was studied as a probable important factor causing such instability. Methods of preparation of two polymorphs, two hydrates, two solvates, and an amorphous form of succinylsulfathiazole are described. Characterization of these forms was carried out using IR spectroscopy and X-ray crystallography. The interconversion of the crystal forms under various physical conditions was studied. The kinetics of transformation of Form I to the water-stable Form II in aqueous suspensions also are discussed. The half-life value of the transformation obtained from an Arrhenius plot ($\simeq$14 hr) was in fair agreement with the experimental value ($\simeq$16 hr) determined for suspensions stored at room temperature.

Keyphrases □ Succinylsulfathiazole—preparation, characterization, and interconversion of different crystal forms, kinetics, Arrhenius plots □ Polymorphism—preparation, characterization, and interconversion of succinylsulfathiazole crystal forms, kinetics, Arrhenius plots □ Crystal forms, succinylsulfathiazole—preparation, characterization, and interconversion

Aqueous suspensions of succinylsulfathiazole prepared from different solid batches were found to vary considerably in their physical stability. Some suspensions exhibited caking, crystal growth, difficult resuspendability, and cement-like precipitate formations which made the suspensions unsatisfactory for use. The physical stability of pharmaceutical preparations has been shown to be affected by the polymorphism of drugs in the same way as their biological availability is altered (1-6). The polymorphism of succinylsulfathiazole is examined in this report as being a probable important element of the physical stability of aqueous suspensions of this drug.

Succinylsulfathiazole was reported to be dimorphic, and methods of preparation of the two crystal forms were described previously (7). Shefter and Higuchi (4) also described methods of preparation of an anhydrous form, three hydrates, and a pentanol solvate of succinylsulfathiazole. Later, Mesley and Houghton (8) described the preparation of four crystal forms and an amorphous form of the same compound. Uncertainty concerning the number of crystal forms of this compound and the lack of reproducibility in preparing and characterizing them were observed. A more thorough investigation of this polymorphic system seemed necessary.

The present work is concerned with the preparation, characterization, interconversion, and kinetics of transformation of the various crystal forms of succinylsulfathiazole.

EXPERIMENTAL[1] AND RESULTS

Materials—Three commercial samples of succinylsulfathiazole[2] were used during this investigation. The purity of the starting materials and the products of crystallization was checked by paper chromatography using the solvent system described by Steel (9). Solvents used for crystallization were of USP or BP quality.

Preparation of Crystal Forms—The general procedure for the preparation of the different crystal forms involved crystallization from specific solvents. For this purpose, 0.2 g of the drug was dissolved in a suitable volume of an appropriate solvent to form a saturated solution at the boiling point of that solvent. The solution was allowed to cool slowly and stand at room temperature until most of the solid crystallized out. The crystals were then separated by filtration through a sintered-glass disk[3], dried in a current of air at room temperature (25°), and stored in a desiccator. Crystals prepared from aqueous solvents had to be dried between two filter papers. Optimum conditions for the preparation of the different crystal forms are summarized as follows.

[1] IR spectra were measured with a Perkin-Elmer double-beam grating IR spectrophotometer, model 237 B. X-ray diffraction measurements were made with a General Electric XRD-6 diffractometer under the following conditions: 3° beam slit and 0.2° detector slit, nickel-filtered CuKα radiation (30 kv, 11 mamp). A Unicam SP500 UV spectrophotometer was used to determine the molar absorptivity of the solution of the various crystal forms.

[2] Boots, England, Katwuk, Holland, and a Chinese brand, all supplied by Chemical Industries Development, Guiza, Egypt.

[3] Jena 39G3.