Table IV—pK_{app} Values per Carbon Atom in Alkyl Sulfates of Some Esters of *N*-Methylquinolinium Acids

N-Methyliodides of	pK_{app} per Carbon Atom in Alkyl Sulfates
2-Ethyl ester quinoline carboxylic acid	0.47
3-Ethyl ester quinoline carboxylic acid	0.45
4-Ethyl ester quinoline carboxylic acid	0.46
5-Ethyl ester quinoline carboxylic acid	0.57
6-Ethyl ester quinoline carboxylic acid	0.43
6-Methyl ester quinoline carboxylic acid	0.44
6-Propyl ester quinoline carboxylic acid	0.50
6-Butyl ester quinoline carboxylic acid	0.50
3-Propyl ester quinoline carboxylic acid	0.52
3-Acetamido ester quinoline carboxylic acid	0.45

the Hammett σ constant, was useful in evaluating the lipohydrophilic character of a molecule upon which biological activity is dependent (Eq. 2).

By using the quinolinium methyliodide as P_H and the different esters of quinolinium acids as P_x, values for π were calculated (Table III).

The change in pK_{app} per carbon atom in the alkyl sulfates of the esters of the quinolinium acid derivatives was found to have an average value of 0.48 (range 0.43–0.57) (Table IV), which is in reasonable agreement with the value of 0.44 for each CH_2 unit reported by Hansch *et al.* (11) and with the value of 0.46 reported by Plakogiannis (7).

REFERENCES

(1) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill, New York, N.Y., 1940.

(2) D. R. Brown, *Quart. Rev. Chem. Soc.*, **6**, 63(1952).

(3) H. H. Jaffe and O. G. Doak, *J. Amer. Chem. Soc.*, **77**, 4441(1955).

(4) C. R. Elderfield and M. Siegel, *ibid.*, **73**, 5622(1951).

(5) E. Bociocchi and G. Illuminati, *Gazz. Chim. Ital.*, **87**, 981(1957).

(6) A. Bryson, *J. Amer. Chem. Soc.*, **82**, 4871(1960).

(7) F. M. Plakogiannis, *Pharm. Acta Helv.*, **46**, 236(1971).

(8) F. M. Plakogiannis and E. A. Lien, *Acta Pharm. Suecica*, **11**, 191(1974).

(9) C. Hansch, P. P. Maloney, T. Fujita, and R. W. Muir, *Nature*, **194**, 178(1962).

(10) C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, F. Geiger, and M. Streich, *J. Amer. Chem. Soc.*, **85**, 2817(1963).

(11) C. Hansch and T. Fujita, *ibid.*, **86**, 1616(1964).

(12) I. Vogel, "Practical Organic Chemistry," 3rd ed., Wiley, New York, N.Y., 1962, p. 543.

(13) J. A. Biles, F. M. Plakogiannis, B. J. Wong, and P. M. Biles, *J. Pharm. Sci.*, **55**, 909(1966).

(14) F. M. Plakogiannis, A. Gringauz, and J. Polak, *Pharm. Acta Helv.*, in press.

(15) F. M. Plakogiannis, E. J. Lien, C. Harris, and J. A. Biles, *J. Pharm. Sci.*, **59**, 197(1970).

(16) G. J. Divatia and J. A. Biles, *ibid.*, **50**, 916(1961).

(17) R. C. Hall and J. A. Biles, *ibid.*, **53**, 869(1964).

(18) A. Albert and J. N. Phillips, *J. Chem. Soc. (London)*, **1956**, 1294.

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Oral Absorption Efficiency of Acid-Labile Antibiotics from Lipid–Drug Delivery Systems

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Abstract □ The utility of cholesterol, cholesteryl acetate, and β-sitosterol in protecting and improving the oral absorption efficiency of acid-labile antibiotics is discussed. The potassium salts of penicillin G and penicillin V and erythromycin lactobionate were studied. The stability of the two penicillins in simulated gastric fluid was determined iodometrically. The rank order of acid protective activity was: cholesteryl acetate > β-sitosterol > cholesterol. Oral administration of erythromycin lactobionate coated with cholesteryl acetate produced a twofold increase in human urinary excretion of erythromycin when compared with the uncoated material. Potassium salts of penicillin G and penicillin V coated with cholesteryl acetate yielded 1.6- and 2-fold higher urine levels, respectively, as compared with the uncoated candidates.

Keyphrases □ Antibiotics, acid labile—oral absorption efficiency of penicillin G potassium, penicillin V potassium, and erythromycin lactobionate from cholesterol, cholesteryl acetate, and β-sitosterol protective carriers □ Penicillins G and V (potassium)—oral absorption efficiency from lipid–drug delivery systems □ Erythromycin lactobionate—oral absorption efficiency from lipid–drug delivery systems □ Lipid–drug delivery systems—oral absorption efficiency of penicillin G potassium, penicillin V potassium, and erythromycin lactobionate from cholesterol, cholesteryl acetate, and β-sitosterol protective carriers.

Solutions of penicillin G lose 50% of their potency in less than 1 min at pH 1 and in about 9 min at pH 2 (1). Solutions of penicillin V in 0.1 *N* hydrochloric acid at 37° were reported to have a half-life of 29 min (2). Chemical inactivation of penicillin G in the gastric fluid has been reported to be responsible for the

Table I—Exposure of 100,000 Units of Plain and Lipid-Coated Penicillin G and Penicillin V to Simulated Gastric Fluid for 1 hr at 37°

Samples Exposed	Penicillin Activity Retained, units[a]		
	Units in Filtrate	Units in Residue	Percent[b] Retained
Potassium penicillin G			
Plain	18.8	—	0.47 ± 0.08
Plus cholesterol (1:1)	70	520.4	14.76 ± 2.01
Plus cholesterol (1:3)	52	657.8	17.74 ± 2.68
Plus cholesterol (1:6)	46	2259.6	57.67 ± 3.54
Plus cholesteryl acetate (1:1)	103	1507	40.27 ± 1.96
Plus cholesteryl acetate (1:3)	92	1798	47.26 ± 1.08
Plus cholesteryl acetate (1:6)	78	2716	69.85 ± 2.15
Plus β-sitosterol (1:1)	110	615.4	18.13 ± 1.56
Plus β-sitosterol (1:3)	86	847.7	23.31 ± 4.28
Plus β-sitosterol (1:6)	82	2328	60.25 ± 1.98
Potassium penicillin V			
Plain	532	—	13.30 ± 0.96
Plus cholesterol (1:1)	602	1121	43.07 ± 1.43
Plus cholesterol (1:3)	554	1709	56.15 ± 2.36
Plus cholesterol (1:6)	483	2320	70.10 ± 4.07
Plus cholesteryl acetate (1:1)	263	2241	67.60 ± 2.69
Plus cholesteryl acetate (1:3)	243	3010	81.33 ± 2.02
Plus cholesteryl acetate (1:6)	197	3135	83.30 ± 3.78
Plus β-sitosterol (1:1)	302	2058	58.99 ± 1.34
Plus β-sitosterol (1:3)	265	2755	75.50 ± 2.22
Plus β-sitosterol (1:6)	231	2867	77.45 ± 3.65

[a] Each value is an average of three determinations. [b] Mean value ±*SD*.

poor oral availability of this antibiotic. Attempts to minimize its destruction in the stomach by the use of enteric-coated preparations have failed since penicillin G is poorly absorbed beyond the duodenum (3). Juncher and Raaschou (4) conducted classic *in vitro* and *in vivo* studies with various salts of penicillin V and sodium penicillin G. The superior acid stability of the former was clearly indicated in dissolution studies at pH 2. The higher blood levels obtained after oral administration of potassium penicillin V could be explained on the basis of this greater acid stability.

Vicek and Vondracek (5) reported that various concentrations of stearic acid did not influence the oral absorption of penicillin V. However, these investigators reported that the addition of 2% stearic acid to tablets containing the potassium salt of penicillin G caused a fivefold increase in blood levels.

Solutions of erythromycin are highly unstable at gastric pH (6, 7). Degradation half-lives of less than 2 min have been reported for solutions of erythromycin and several of its ester derivatives (8). Laboratory and clinical investigations have shown that the biological availability of a series of erythromycin esters was inversely proportional to their dissolution rate in 0.1 *N* hydrochloric acid (9, 10). Approximately 2–5% of an orally administered dose was excreted in the urine whereas 12–15% was found in the urine after an intravenous dose (11).

The water-soluble lactobionate salt is suitable for intravenous administration. The stability of this salt has been studied (12).

The GI absorption of drugs administered in lipid-containing dosage forms has received little attention. Recently, Carrigan and Bates (13) reported that an oil-in-water emulsion of griseofulvin was absorbed 2.5-fold better than an aqueous suspension. Earlier studies in this laboratory (14) showed that the rate and extent of salicylate oral absorption were not impaired even when large excesses of cholesterol, cholesteryl acetate, or β-sitosterol were present. On the other hand, these lipid-coated salicylates exhibited slow rates of dissolution in simulated GI fluids at 37°. Such a marked difference between the *in vitro* dissolution and the *in vivo* rate of absorption was surprising. Cholesterol in cholesterol–hormone

Table II—Urine Recovery of Erythromycin in Two Human Adults after Oral Administration of Various Erythromycin Samples (500,000 μg of Activity)

Sample Administered	Subject	Erythromycin Activity Recovered in the Urine, μg			Percent Excreted
		0–12 hr	12–24 hr	Total 24 hr	
Erythromycin stearate film-coated tablet	C.I.J.	14,538	4,456	18,994	3.80
	S.P.P.	1,372	—[a]	1,372	0.27
Erythromycin lactobionate	C.I.J.	20,064	—[a]	20,064	4.01
	S.P.P.	—[a]	—[a]	—[a]	—[a]
Erythromycin lactobionate plus cholesteryl acetate (1:6)	C.I.J.	29,120	2,064	31,184	6.24
	S.P.P.	2,775	4,370	7,145	1.43

[a] Available data do not permit calculation since the erythromycin activity was less than the standard used.

mixtures enhanced the permeation of the latter through lipoidal membranes (15, 16).

These observations led to the present investigation of the utility of cholesterol, cholesteryl acetate, and β-sitosterol as protective carriers for acid-labile antibiotics. The antibiotics selected were the potassium salts of penicillin G and penicillin V and erythromycin lactobionate.

EXPERIMENTAL

Materials—The following were used: potassium benzylpenicillinate[1] (USP), potassium phenoxymethylpenicillinate[1] (USP), erythromycin lactobionate[2] USP, cholesterol[3] USP, cholesteryl acetate[3], β-sitosterol[3], erythromycin stearate film-coated tablets[2] USP, iodine solution[4] (0.1 *N*), soluble starch[4], sodium thiosulfate solution[4] (0.1 *N*), chloroform[5] NF, sodium hydroxide[5] (reagent grade), potassium phosphate[4], anhydrous monobasic (reagent grade), potassium phosphate[4] (anhydrous dibasic, reagent grade), and hydrochloric acid[6] (reagent grade).

Equipment—The following were used: a constant-temperature shaker bath[7], a pH meter[8], a microburet (50 divisions/ml), a gyrotory incubator shaker[9], brass sieves[10], an immersion filter[11] [1.7 × 5.1 cm (0.7 × 2 in.), medium porosity], a Swinny adaptor[12] (13 mm), and a filter disk[12] (13-mm diameter, 0.45 μm).

Preparation of Lipid-Coated Penicillins—Samples for the *in vitro* stability and *in vivo* bioavailability studies were prepared in drug–lipid ratios of 1:1, 1:3, and 1:6 by the following procedure. A weighed quantity of potassium penicillin G (252.36 mg = 400,000 units) or potassium penicillin V (266.66 mg = 400,000 units) was placed in a glass mortar. A weighed quantity of cholesterol, cholesteryl acetate, or β-sitosterol for an appropriate ratio was dissolved in a minimum quantity of chloroform to produce a saturated solution of lipid in chloroform. A 2-ml aliquot of this solution was poured onto the penicillin powder and gently triturated with a pestle.

During this procedure, a gentle flow of nitrogen gas was introduced to accelerate the evaporation of the chloroform. The sample was dried to a semisolid consistency. This procedure was repeated until the required quantity of lipid had been added. The final mixture of penicillin and lipid was doughy in consistency. The doughy mass was pressed through a 20-mesh sieve to form small granules. These granules were dried in a current of nitrogen and then dried[13] overnight at 37°. The dried granules were passed through a 20-mesh sieve and caught on a 40-mesh sieve. The 20–40-mesh fraction of penicillin–lipid granules was used for the *in vitro* stability and *in vivo* oral absorption studies.

Preparation of Lipid-Coated Erythromycin—Lipid-coated erythromycin lactobionate was prepared for an *in vivo* study as follows. A vial of erythromycin lactobionate powder for intravenous use containing 1 g of erythromycin activity and 180 mg of benzyl alcohol was quantitatively transferred to a glass mortar. To this powder mixture was added 6 g of cholesteryl acetate in chloroform in a similar manner to that already described. Dried, lipid-coated granules, 20–40 mesh, were obtained.

Procedure for *In Vitro* Stability Testing of Various Lipid-Coated Penicillins—An amount of granules corresponding to 100,000 units of penicillin activity was added to 25 ml of simulated gastric fluid (USP XVIII) without pepsin (pH 1.2) at 37° contained in 120-ml (4-fluid oz) amber-colored bottles. The bottles were tightly screw capped and kept in a 37° constant-temperature water bath with a reciprocating motion (stroke 5 cm, 100 times/min). After 1 hr, the undissolved penicillin was filtered off. The filtrate was passed through a 0.45-μm filter and assayed for penicillin by a modified iodometric method (17).

The residue remaining after filtration was dried in an oven at 37° overnight. The residue was dissolved in 10 ml of chloroform, and the chloroform solution was extracted with four 10-ml portions of pH 6 phosphate buffer. The four phosphate buffer extractions were combined and assayed iodometrically for penicillin (Table I).

Bioavailability of Erythromycin from Lipid-Coated Granules and Film-Coated Tablets—Two healthy adult male subjects were used for a bioavailability study of lipid-coated erythromycin lactobionate granules following oral administration after an overnight fast. Urine samples were pooled to represent collection intervals of 0–12 and 12–24 hr and were kept frozen in dry ice until they were analyzed for erythromycin microbiologically[14]. The following preparations were compared in a crossover study: erythromycin stearate film-coated tablets[2], 500 mg of activity; erythromycin lactobionate powder, 500 mg of activity; and erythromycin lactobionate granules containing cholesteryl acetate (1:6), 500 mg of activity (Table II).

Bioavailability of Penicillin G and Penicillin V from Lipid-Coated Granules—Initial screening for the bioavailability of the penicillins was conducted in two healthy human adults to ascertain the best lipid-coated candidates for the larger panel. The two subjects were dosed with 400,000 units of uncoated penicillin after an overnight fast and were instructed to fast an additional 3 hr after the dose was administered. Pooled urine samples represented 0–4- and 4–8-hr collections. Urinary recovery of penicillin from the test preparations was determined microbiologically using *Sarcina lutea* (ATCC-9341) as the test organism (17). Each of the three lipids, at an antibiotic–lipid ratio of 1:6, was then crossed over in the same two individuals (Table III).

In a similar fashion, a panel of six human adults was used to compare the bioavailability of the two penicillins coated with cholesteryl acetate (1:6) (Table IV).

Table III—Antibiotic Urine Recovery in Two Adults after Oral Administration of Plain and Lipid-Coated Potassium Salts of Penicillin G and Penicillin V (Single Dose of 400,000 Units)

	Urine Recovery, %			
	Subject C.I.J.		Subject S.P.P.	
Sample Administered	0–4 hr	4–8 hr	0–4 hr	4–8 hr
Potassium penicillin G				
Plain	19.95	3.99	18.20	1.89
Plus cholesterol (1:6)	25.11	7.01	22.84	4.10
Plus cholesteryl acetate (1:6)	30.11	8.70	25.03	8.91
Plus β-sitosterol (1:6)	29.94	3.00	24.90	2.96
Potassium penicillin V				
Plain	29.16	5.10	22.40	4.09
Plus cholesterol (1:6)	32.08	11.02	31.06	8.89
Plus cholesteryl acetate (1:6)	40.79	18.23	39.98	14.12
Plus β-sitosterol (1:6)	35.74	9.00	37.00	6.01

RESULTS

In Table I it can be seen that penicillin V is more stable than penicillin G in simulated gastric fluid. After 1 hr, 13.3 ± 0.96% of penicillin V was retained whereas only 0.47 ± 0.08% of penicillin G remained. The stabilizing effect of the lipid coatings is also apparent. As the lipid concentration is increased, the penicillin activity in the filtrate is diminished and the potency retained in the residue is increased. For example, in going from antibiotic–lipid ratios of 1:1, 1:3, and 1:6 in the case of the potassium salt of penicillin G–cholesteryl acetate, the filtrates contained 103, 92, and 78 units,

[1] Pfizer Inc., New York, N.Y.
[2] Abbott Laboratories, North Chicago, Ill.
[3] K and K Laboratories Inc., Plainview, N.Y.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] Matheson, Coleman and Bell, Norwood, Ohio.
[6] J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Model W.B.R., New Brunswick Scientific Co., New Brunswick, N.J.
[8] Beckman Zeromatic, Beckman Instrument Co., Fullerton, Calif.
[9] Model G-27, New Brunswick Scientific Co., New Brunswick, N.J.
[10] U.S. Series designation [12.7 cm (5 in.) diameter]: No. 10, 2000 μm (9 mesh); No. 14, 1410 μm (12 mesh); No. 18, 1000 μm (16 mesh); No. 35, 500 μm (32 mesh); No. 60, 250 μm (60 mesh); and No. 140, 105 μm (150 mesh); Arthur H. Thomas Co., Chicago, Ill.
[11] Arthur H. Thomas Co., Chicago, Ill.
[12] Millipore Corp., Bedford, Mass.
[13] Drierite.
[14] The microbiological assays were conducted by Ruth Wallace, Pfizer Inc.

Table IV—Antibiotic Urine Recovery (Percent) in Six Adults after Oral Administration of Plain and Cholesteryl Acetate-Coated (1:6) Potassium Salts of Penicillin G and Penicillin V (Single Dose of 400,000 Units)[a]

	Potassium Penicillin G				Potassium Penicillin V			
	Plain		Lipid Coated		Plain		Lipid Coated	
Subject	0–4 hr	4–8 hr	0–4 hr	4–8 hr	0–4 hr	4–8 hr	0–4 hr	4–8 hr
S.P.P.	18.20	1.89	25.11	8.91	22.40	4.09	39.97	14.21
C.I.J.	21.78	3.09	30.11	8.70	29.16	5.10	40.79	18.24
F.S.	19.73	3.84	26.87	10.12	29.02	3.06	40.11	16.62
R.P.K.	20.82	3.74	28.82	10.57	31.82	3.97	42.29	19.09
M.S.	17.73	2.86	26.91	9.08	30.23	3.30	44.35	13.50
E.A.	16.14	2.98	21.97	7.83	26.15	4.09	37.27	16.92
Average	19.07	3.07	26.63	9.21	28.12	3.94	40.80	16.26
$\pm SD$	4.5	1.3	5.5	1.9	6.8	0.7	4.5	4.3

[a] Statistical evaluation of the data by the Student t test gave the following p values: potassium penicillin G, plain *versus* coated, 0–4 hr, $p < 0.05$, and 4–8 hr, $p < 0.001$; potassium penicillin V, plain *versus* lipid coated, 0–4 hr, $p < 0.01$, and 4–8 hr, $p < 0.001$.

respectively. The antibiotic potency retained in the residues, as anticipated, increased (1507, 1798, and 2716 units, respectively).

Cholesteryl acetate was the most protective of the three lipids studied. It was not surprising to find that the oral absorption efficiency for both the penicillins was best when cholesteryl acetate was employed as the lipid coating (Table III). The urine recovery data from a six-patient crossover study are shown in Table IV. The higher antibiotic potency recovered was statistically significant for either penicillin coated with cholesteryl acetate (1:6) when compared with the urine levels obtained with uncoated material.

In Table II it can be seen that the acid-labile erythromycin lactobionate yielded higher antibiotic urine levels when coated with cholesteryl acetate in a ratio of 1:6. In addition, for both subjects there was a greater urine recovery of erythromycin after oral administration of the lipid-coated lactobionate as compared with erythromycin stearate.

Thus, it has been shown that the acid-labile antibiotics, penicillin G, penicillin V, and erythromycin, can have their oral availability enhanced by the application of a protective lipid coating such as cholesteryl acetate. Furthermore, the significantly higher 4–8-hr urine recoveries of antibiotic from the lipid-coated penicillins are indicative of a more prolonged systemic action. It appears from the presented data that further exploration of the utility of lipids as drug delivery systems is warranted.

REFERENCES

(1) R. Broderson, "Inactivation of Penicillin in Aqueous Solution," Einar, Munksgaard, Copenhagen, Denmark, 1949, p. 104.

(2) A. Forist, L. Brown, and M. Royer, *J. Pharm. Sci.*, **54**, 476(1965).

(3) T. H. Lin, J. R. Guarini, S. P. Eriksen, and J. V. Swintosky, *ibid.*, **53**, 1357(1964).

(4) H. Juncher and F. Raaschou, *Antibiot. Med. Clin. Ther.*, **4**, 497(1957).

(5) V. Vicek and M. Vondracek, *Farmakoter. Zpr.*, **5**, 161(1959).

(6) "AMA Drug Evaluations," 1st ed., American Medical Association, Chicago, Ill., 1971, p. 401.

(7) H. W. Murphy, "Antibiotics Annual, 1953–1954," Medical Encyclopedia Inc., New York, N.Y., 1953, pp. 500–513.

(8) V. C. Stephens, J. W. Conine, and H. W. Murphy, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 620(1959).

(9) E. Nelson, *Chem. Pharm. Bull.*, **10**, 1099(1962).

(10) V. C. Stephens and J. W. Conine, "Antibiotics Annual, 1958–1959," Medical Encyclopedia Inc., New York, N.Y., p. 346.

(11) "The Pharmacological Basis of Therapeutics," 3rd ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1965, p. 1266.

(12) H. W. Unterman, E. Constantin, and M. Rusan, *Farmacia (Bucharest)*, **12**, 625(1965).

(13) P. J. Carrigan and T. R. Bates, *J. Pharm. Sci.*, **62**, 1476(1973).

(14) S. P. Patel, Ph.D. dissertation, St. John's University, Jamaica, N.Y., 1974.

(15) R. S. Snart and M. J. Wilson, *Nature*, **215**, 954(1967).

(16) R. S. Snart, *ibid.*, **215**, 957(1967).

(17) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics," Medical Encyclopedia Inc., New York, N.Y., 1955, pp. 15, 17, 97.

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