data acquisition. The data with which the system precision of the dosage form analysis was compared by various quantification techniques is presented in Table II. The precision obtained by any of the quantification techniques was quite acceptable. Since external standard techniques require fewer calculations and are more convenient in terms of sample preparation, those laboratories in which automated equipment is used may select this as the preferred quantification technique.

Two different quantification techniques were used when the method was applied to drug substance stability studies. In such studies, the amount of carprofen in each sample was determined by using internal standard calculations similar to those used in the dosage form assay. However, the quantifications performed to estimate the impurity and degradation product levels were carried out by using an area percent calculation method corrected for the presence of the internal standard in the chromatogram. This technique has been found to be particularly useful when conducting drug substance stability studies on compounds in early phases of development. An underlying assumption with such a quantification technique is that potential degradation products and impurities have the same absorptivity as the parent compound at the chosen detector wavelength. Although this assumption may not always be valid, in many cases it is reasonable. It eliminates the need for reference standards of all impurities and degradation products, which are usually not available during the early stages of development. Considering this restriction, the technique described above is certainly acceptable for demonstrating stability trends. When reference materials become available, the data generated by using the area percent method can be refined or recalculated to take into account any differences in absorptivity. The area percent technique is also useful if an outside laboratory (without access to reference standards of potential impurities and degradation products) is conducting the assay.

The method described has been shown to be accurate, precise, and broadly applicable to all control and analysis laboratories and functions. The sample preparations are simple, and the method can easily be automated.

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Development and Evaluation of Enteric-Coated Penicillamine Tablets

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Abstract □ Commercially available 250-mg penicillamine tablets were converted into enteric-coated tablets. Based on *in vitro* dissolution and disintegration tests, tablets coated with five layers of a cellulose acetate phthalate formulation by a modified pan coating technique were judged to be superior to other coated tablets. These tablets resisted disintegration in simulated gastric fluid over a 4-h period and disintegrated in an average of 21 min in simulated intestinal fluid. Enteric-coated penicillamine tablets were tested *in vivo* in nine weanling pigs divided into three groups: a negative control group, a test group dosed with enteric-coated penicillamine tablets, and a positive control group dosed with uncoated tablets. The incidence of GI tract bleeding, as determined by daily occult blood tests of the stools, was significantly less in the animals receiving the enteric-coated tablets when compared with the positive control group. The enteric-coated dosage form appeared to decrease GI tract irritation caused by penicillamine. Plasma concentration–time curves for penicillamine in the pigs were similar in shape to those reported in humans. Atypical double peaks occur in both species. Relative bioavailability of the enteric-coated tablet was found to be 67% when compared with the uncoated tablet. This apparent reduction is probably due to a large intrasubject variation in areas under the plasma concentration–time curves and not to a dosage form effect.

Keyphrases □ Penicillamine—development and evaluation of enteric-coated tablets □ Formulations—penicillamine, development and evaluation of enteric-coated tablets

Penicillamine has been used for many years in the treatment of Wilson's disease and cystinuria. More recently, the drug has been approved for the treatment of rheumatoid arthritis. Due to severe adverse reactions, penicillamine therapy in the arthritic patient is normally limited to those who have been previously treated unsuccessfully by conventional therapy (1).

It has been estimated that up to one-third of the patients

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Table I—Enteric Coating Formulations

Formulation Ingredients	% w/w
Cellulose acetate phthalate coating	
Cellulose acetate phthalate	12.0
Diethyl phthalate	3.0
Ethyl acetate	42.5
Isopropyl alcohol	42.5
Polyvinyl acetate phthalate coating	
Polyvinyl acetate phthalate	10.0
Polyethylene glycol 400	1.0
Methanol	89.0

treated orally with penicillamine must discontinue use because of adverse reactions such as gastric distress (2). Gastric upset, which is normally decreased by coadministering food or antacids with the drug, is not possible in this case because the drug chelates magnesium, calcium, and iron (1). In previous work, it has been shown that plasma levels of the unchanged drug are reduced in humans when penicillamine is given with a meal (3).

Enteric-coated dosage forms release drugs in the intestine and have been shown to decrease gastric irritation (4). In this study, an enteric-coated penicillamine tablet is described, and this dosage form is evaluated *in vitro* and *in vivo* in weanling pigs.

EXPERIMENTAL SECTION

Materials– Penicillamine tablets[1], cellulose acetate phthalate[2], and polyvinyl acetate phthalate[3] were obtained commercially. Simulated gastric and intestinal fluids were made as directed in USP XX (5).

Coating Methods– *Dip Coating*—The capsule-shaped tablet was held at one end with tweezers and dipped a little over one-half way into 50 mL of 10% w/w cellulose acetate phthalate in acetone. The tablet was withdrawn after 10 s, and its free end was touched to the lip of the beaker to remove excess solution. The tablet was held in the air for 2 min to dry. The procedure was repeated until both halves of the tablet were coated three times. Twenty tablets were coated by this procedure. The tablets were placed in an evaporating dish and dried at 50°C for 16 h.

Pan Coating—A 15-cm diameter stainless steel coating pan was used. Two aluminum baffles were mounted in the pan to promote a smooth tumbling action of the tablets. The 11.4 × 0.64-cm baffles extended from the inside of the pan rim to 3.8 cm from the bottom center. They were equally spaced, 180° apart. The baffles were bent to fit the curvature of the pan and sloped in the direction of the pan rotation. The pan was rotated at 32 rpm.

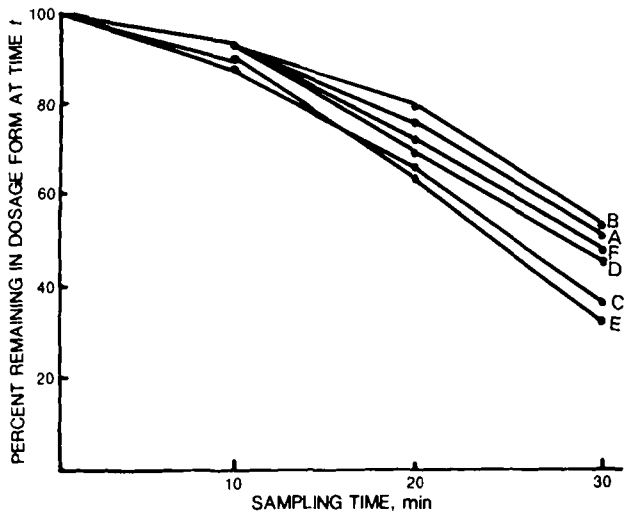

Figure 1—*Effect of storage at 50°C on the dissolution of enteric-coated penicillamine tablets at pH 6.0. Key to storage times: (A) 0 d; (B) 15 d; (c) 30 d; (D) 45 d; (E) 60 d; (F) 75 d.*

[1] Depen (250 mg); Wallace Laboratories.
[2] Eastman Kodak.
[3] Colorcon, Inc.

Table II—Mean Disintegration Time for Enteric-Coated and Uncoated Tablets as Compared with Coat Thickness[a]

Tablet Group	Coat Thickness, cm	Disintegration Time, min	
		Gastric Fluid[b]	Intestinal Fluid
Uncoated	0.000	28	21
Cellulose acetate phthalate			
5 coats	0.010	>60 (*b*)	20
7 coats	0.012	>60 (*a*)	20
10 coats	0.013	>60 (*a*)	22
Polyvinyl acetate phthalate			
5 coats	0.007	>60 (*d*)	13
10 coats	0.010	>60 (*c*)	16
15 coats	0.013	>60 (*c*)	17
20 coats	0.018	>60 (*b*)	21
25 coats	0.023	>60 (*b*)	14
30 coats	0.030	>60 (*a*)	18

[a] $n = 6$. [b] Key: (*a*), no damage to coat; (*b*), some softening of coat and slight pitting; (*c*), obvious coat damage, deep pits; (*d*), partial tablet core disintegration.

Table I contains the two formulations that were applied to the tablets by the pan coating procedure. The cellulose acetate phthalate formulations (200 g) were prepared by mixing 95.0 mL of ethyl acetate and 180.6 mL of isopropyl alcohol in a 600-mL beaker. The solution was vigorously stirred with a magnetic stirrer, and 24.0 g of cellulose acetate phthalate was dissolved in the solution followed by the addition of 2.7 mL of diethyl phthalate; the solution was continuously stirred for 20 min.

The polyvinyl acetate phthalate formulation was prepared by slowly adding 10.0 g of polyvinyl acetate phthalate to 75.0 mL of methanol over a 15-min period. The solution was vigorously stirred with a magnetic stirrer. After the polyvinyl acetate phthalate was added, 0.9 mL of polyethylene glycol 400 was added, and the solution was stirred for 10 min. The weight of the solution was adjusted to 100.0 g by the addition of methanol. The solution was stirred for an additional 10 min.

A typical batch of 90 tablets was placed in a 1000-mL beaker, and 3 mL of the formulation was added. The tablets were stirred by hand for 1 min and then transferred to the pan. The pan was rotated for 10 min at 32 rpm. The tablets were stirred by hand during the first 2 min of rotation to prevent clumping. When the coat was dry, the tablets were returned to the 1000-mL beaker. The procedure was repeated until the required number of coats were applied. After receiving the final coating, the tablets were placed on a drying tray in a 50°C oven for 16 h. The number of coats used were 5, 7, and 10 for the cellulose acetate phthalate formulation and 5, 10, 15, 20, 25, and 30 for the polyvinyl acetate phthalate formulation.

***In Vitro* Tests of Enteric-Coated Tablets**—*Uniformity Test*—Tablets from each batch were visually inspected for uniformity in appearance, smoothness of coat, and absence of cracks. Twenty tablets in each batch were individually weighed, and the mean weight and *SD* were determined.

A micrometer was used to determine the average length, width, and thickness of the 20 tablets from each batch. The mean thickness of the enteric coat was calculated by:

$$\text{Coat Thickness} = \frac{(L_c - L_n) + (W_c - W_n) + (T_c - T_n)}{(2) \cdot (3)}$$

where L_c is the length of coated tablet, W_c is the width of coated tablet, T_c is the thickness of coated tablet, L_n is the length of uncoated tablet, W_n is the width of uncoated tablet, and, T_n is the thickness of uncoated tablet. The numerator of the equation sums the differences between the coated and uncoated tablets in three dimensions (length, width, and thickness). The denominator contains two correction factors: the three dimensions and the two sides of the tablet contained in each measurement.

Disintegration Test—The USP XX (6) disintegration test for enteric-coated tablets was used to evaluate the penicillamine tablets coated by either the dip coating or pan coating procedure. A standard six-tube basket-rack assembly[4] was used for all the determinations. After 1 h of operation using simulated gastric fluid as the disintegration fluid, the basket was lifted from the fluid, and the tablets were inspected for signs of disintegration, cracking, or softening. The condition of the coated tablets was rated by the following scale: (*a*) no obvious damage, (*b*) some softening of the coat and slight pitting, (*c*) obvious coat damage, deep pits, (*d*) partial tablet core disintegration, and (*e*) total tablet disintegration. The medium was then changed to simulated

[4] USP disintegration apparatus; Fisher Scientific Co.

Table III—Dissolution of Enteric-Coated and Uncoated Tablets at pH 1.2 [a]

Sampling Time, min	Dissolution, % Remaining ± *SD*					
		Cellulose Acetate Phthalate			Polyvinyl Acetate Phthalate	
	Uncoated	5 Coats	7 Coats	10 Coats	25 Coats	30 Coats
5	76.4 ± 6.9	—[b]	—	—	—	
10	65.0 ± 5.2	—	—	—	—	—
15	55.5 ± 7.5	—	—	—	—	—
20	46.3 ± 4.8	—	—	—	—	—
30	38.5 ± 2.9	99.7 ± 0.6	100 ± 0	100 ± 0	97.1 ± 2.8	98.2 ± 1.7
60	—	96.8 ± 3.0	100 ± 0	100 ± 0	91.5 ± 1.3	98.3 ± 0.6
120	—	94.9 ± 2.6	100 ± 0	97.8 ± 3.8	82.6 ± 2.8	96.4 ± 2.8
180	—	92.7 ± 1.3	100 ± 0	97.8 ± 3.2	74.8 ± 4.5	94.5 ± 1.1
240	—	90.0 ± 3.4	98.6 ± 0.6	93.4 ± 5.8	71.1 ± 4.0	92.6 ± 2.8

[a] $n = 3$. [b] No sample taken.

intestinal fluid and disks were added to each tube. The disintegration time for each of the six tablets was noted to the nearest minute.

Dissolution Test—A USP rotating-basket dissolution apparatus[5] was used for all dissolution studies. A single enteric-coated or uncoated tablet was placed into the 40-mesh basket and lowered into the specified medium such that the basket was 2.0 cm from the bottom of the vessel. The basket was rotated at 100 rpm. Each dissolution test was run in triplicate.

Three dissolution media were used. A pH 1.2 solution (0.063 M HCl) was used to simulate gastric fluid. A pH 6.0 sodium phosphate buffer was used to simulate intestinal fluid in the duodenum. A pH 8.0 sodium phosphate buffer was used to simulate intestinal fluid with a pH in the upper range.

A colorimetric assay developed by Mann and Mitchell (7) was used to quantitate penicillamine in the dissolution medium. The absorbance was determined at 412 nm with a spectrophotometer[6]. The amount of penicillamine contained in the sample was determined from a standard curve prepared for penicillamine in each of the dissolution solutions. A linear relationship held over the concentration range tested.

Stability of the Enteric Coat—The effect of storage at different temperatures on the enteric-coated tablet was investigated. Enteric-coated tablets with five layers of the cellulose acetate phthalate formulation, prepared by the pan coating procedure, were stored and tested along with uncoated tablets so that changes in the tablet core could be determined. Samples of each tablet were stored in ~118-mL amber glass prescription bottles at 4°C, 25°C, 37°C, or 50°C. Eighteen tablets were removed from each bottle at various times during a 75-d period for testing.

The USP disintegration test for enteric-coated tablets was performed on six tablets from the enteric-coated group at each sampling period. The tablets were inspected for signs of disintegration, cracking, or softening after 1 h in simulated gastric fluid. The disintegration time in simulated intestinal fluid was then noted to the nearest minute. The disintegration time of six uncoated tablets in simulated intestinal fluid was determined to the nearest minute.

The procedure described above was used to test the dissolution of the coated and noncoated tablets at pH 1.2 and 6.0. Samples were removed at 0, 10, 20, and 30 min from the pH 6.0 solution and at 0, 30, and 60 min from the pH 1.2 solution. The concentration of penicillamine in each dissolution medium was determined by colorimetry, as described above. Each test was performed on three tablets.

Experimental Animals—Nine disease-free, acclimated, Yorkshire pigs (5–8 weeks old) were randomly divided into three groups by using color-coded tags. Control pigs, pigs receiving uncoated penicillamine tablets, and pigs receiving enteric-coated penicillamine tablets were housed in separate pens and fed ~2.2 kg of commercial hog feed daily. Food was offered for at least 4 h after dosing and was removed at least 12 h before the next dose was given. Each pen was equipped with an automatic watering device which allowed water *ad libitum*.

A dosing syringe was designed to administer the tablets to the pigs. The device consisted of a 26.6-cm flexible plastic tube (0.7 cm i.d. and 1.1 cm o.d.), with a 31-cm, flexible polytef rod (0.6-cm diameter) which was inserted into the tube and used as a plunger. The entire apparatus was bent to promote insertion past the throat of the pig. Before dosing, the plunger and the outside of the tube were coated with a water-soluble lubricant. The penicillamine tablet was placed into the end of the dosing syringe, and the tube was inserted 10–15 cm down the esophagus of the pig, where the tablet was injected. Each pig received several milliliters of 50% w/v sucrose solution before dosing; the mouth was flushed with the sucrose solution after dosing, and a small slice of apple was then given to decrease the incidence of tablet regurgitation. Another tablet was administered if the first one was rejected.

[5] Erweka Dissolution apparatus (type DT); Chemical and Pharmaceutical Industry Co.

[6] Spectronic 20 colorimeter/spectrophotometer; Bausch and Lomb.

The control pigs were treated in an identical fashion except that no tablet was loaded into the dosing apparatus. Dosing occurred daily for a period of 2 months.

Bioavailability Study—The study was conducted on 2 d, 1 week apart. On the first day, one animal from each treatment group was used. The other pigs were dosed as usual for an additional week and then used in the study.

Each animal received an appropriate 250-mg penicillamine tablet (uncoated or enteric coated) and was prepared for surgery immediately after dosing. A catheter was surgically placed in the jugular vein such that the tip was in the anterior vena cava; the catheter was exteriorized behind the base of the ear and attached to a 15-gauge needle, which was taped to the neck to allow access.

Blood samples were obtained at 0.5-h intervals for 12 h. The catheter was aspirated until fresh blood entered the syringe. One milliliter of blood was collected with a fresh 3-mL syringe containing 100 U of heparin, and the catheter was flushed with 2–3 mL of heparinized saline (100 U/mL). The blood was centrifuged at 500×*g* for 10 min. Plasma (500 μL) was transferred to another centrifuge tube, 100 μL of 20% w/v trichloroacetic acid was added, and the contents of the tube were gently mixed. The sample was centrifuged at 500×*g* for an additional 10 min. The protein-free supernatant was decanted and stored at 20°C until assayed; all samples were analyzed within 7 d.

A procedure developed by Bergstrom *et al.* (8) to assay penicillamine in

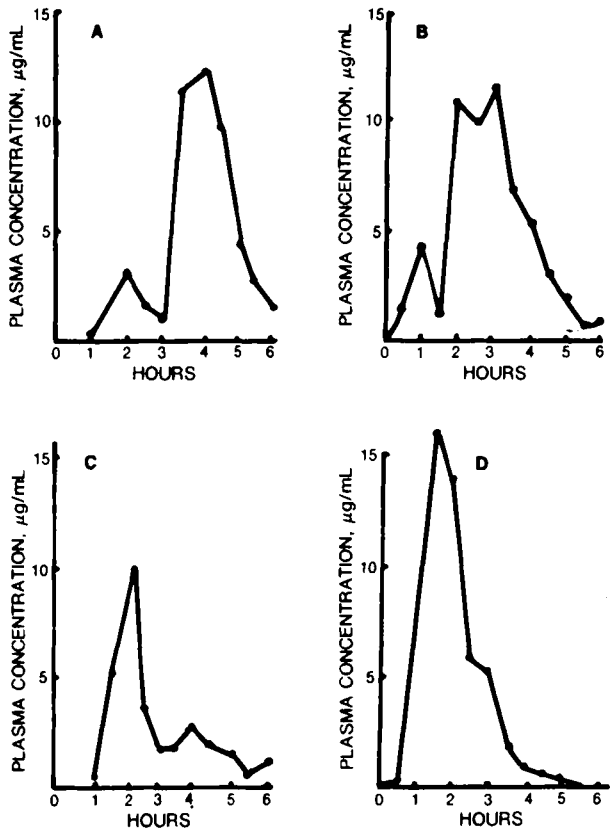

Figure 2—*Plasma concentration–time curves for penicillamine in pigs. Key: (A) pig C28 (19.8 kg); (B) pig B24 (18.5 kg); (C) pig C26 (24.0 kg); (D) pig B23 (29.0 kg).*

Table IV—Dissolution of Enteric-Coated and Uncoated Tablets at pH 6.0 [a]

Group	Dissolution, % Remaining ± *SD*			
	5 min	10 min	15 min	20 min
Uncoated	70.1 ± 5.0	49.5 ± 5.7	31.6 ± 4.0	19.3 ± 3.9
Cellulose acetate phthalate				
5 coats	97.0 ± 2.6	77.5 ± 3.3	58.7 ± 4.0	34.2 ± 9.3
7 coats	97.9 ± 1.8[b]	95.5 ± 2.2[b]	92.0 ± 3.1[b]	84.5 ± 2.0[b]
10 coats	100.0 ± 0.0[b]	97.5 ± 2.3[b]	94.8 ± 4.6[b]	90.3 ± 6.6[b]
Polyvinyl acetate phthalate				
25 coats	88.5 ± 2.0	81.1 ± 2.3[b]	63.3 ± 7.8	46.9 ± 7.0
30 coats	94.2 ± 1.3	85.0 ± 2.6[b]	72.5 ± 7.2[b]	63.5 ± 6.2[b]

[a] $n = 3$. [b] Significantly different from uncoated group at $p = 0.001$.

Table V—Dissolution of Enteric-Coated and Uncoated Tablets at pH 8.0 [a]

Group	Dissolution, % Remaining ± *SD*			
	5 min	10 min	15 min	20 min
Uncoated	75.7 ± 1.9	57.3 ± 3.4	31.8 ± 4.4	20.5 ± 3.0
Cellulose acetate phthalate				
5 coats	80.1 ± 4.8	64.0 ± 7.3	50.7 ± 12.0	37.8 ± 9.4
7 coats	83.7 ± 10.5	67.2 ± 8.6	53.1 ± 3.0	42.5 ± 4.2
10 coats	90.9 ± 4.2	72.8 ± 8.7	56.7 ± 15.6	47.5 ± 15.7
Polyvinyl acetate phthalate				
25 coats	83.7 ± 2.4	67.6 ± 2.5	48.3 ± 5.5	35.0 ± 6.0
30 coats	87.7 ± 1.4[b]	72.4 ± 3.6	56.7 ± 8.0	48.7 ± 7.3

[a] $n = 3$. [b] Significantly different from uncoated group at $p = 0.001$.

plasma with a liquid chromatograph[7], which was equipped with a cation-exchange analytical column[8] and an electrochemical detector[9], was used. The detector consisted of a thin-layer electrochemical cell[10] containing a mercury-gold amalgam working electrode, a glassy carbon auxiliary electrode, and a silver-silver chloride reference electrode. The potential of the working electrode was set at +0.1 V *versus* the reference electrode. The range on the detector was varied from 5 to 20 nA, depending on the sample concentration. The mobile phase consisted of a 0.035 M citric acid–0.005 M disodium phosphate buffer. The buffer was filtered and deaerated before use. Penicillamine has an average retention time of 3.34 min at a flow rate of 2 mL/min.

A standard curve of peak height ratios, prepared with plasma samples obtained from the control animals, was linear. The plasma samples were treated as described above. One previously prepared standard was analyzed with each set of samples. A total of 20 μL of the standard was injected into the liquid chromatograph approximately every 0.5 h during sample analysis. The peak height of an unknown plasma sample was then compared with the average peak height of the standards injected before and after the sample. Duplicate 20-μL injections of each unknown plasma samples were made.

Occult Blood Tests—Stool samples were collected from each pen daily before drug administration. The stool samples were tested for occult blood with a commercially available kit[11]. The incidence of positive tests after 55 d of dosing was determined for each group.

Tissue Sample Collection—Immediately after sacrificing the animals, tissue sections were removed from the GI tract, including glandular and nonglandular stomach, duodenum, jejunum, colon, and a mesenteric lymph node. The tissues were preserved in buffered formalin, embedded in paraffin, sectioned, and mounted on slides. The slides were stained with hematoxylin and eosin for routine histological examination.

RESULTS AND DISCUSSION

Coating Method— Two methods of coating the penicillamine tablets were investigated. The dip coating procedure was slow and tedious, requiring approximately 2 h to coat 20 tablets. Although the coating solution overlapped at the center of the tablets, causing a nonuniform coat, these tablets performed well in the USP disintegration test. No damage to the coat was apparent after 1 h of testing in simulated gastric fluid, and six tablets disintegrated in an average of 20 min in simulated intestinal fluid.

The pan coating procedure provided an excellent method of coating the tablets. The procedure was easy and quick; it produced tablets with uniform coats and proved to be the method of choice.

Evaluation of Coated Tablets—The enteric-coated tablets prepared in this study were judged on ease of preparation, appearance, uniformity, and performance in disintegration and dissolution tests. Tablets coated with polyvinyl acetate phthalate and cellulose acetate phthalate by a pan coating procedure were evaluated.

The polyvinyl acetate phthalate formulation was much more difficult to work with than the cellulose acetate phthalate formulation. Polyvinyl acetate phthalate solutions are tacky and are more suited to a spray coating procedure. Tablets pan-coated with this formulation tended to clump together and to stick to the side of the pan. Tablet sticking can normally be controlled by the use of a dusting powder. Penicillamine, however, chelates with magnesium, calcium, and aluminum, which eliminates the possibility of using common dusting powders, such as magnesium stearate and talc. Tablets coated with cellulose acetate phthalate had clean coats with a high gloss. Due to the tackiness of polyvinyl acetate phthalate, the tablets coated with this polymer were not smooth.

Both formulations provided coated tablets of uniform size and weight. The average dimensions of 20 tablets from each batch had <1% variation in any dimension. The thickness of the coat increased with increasing number of coats.

The results of the disintegration tests are given in Table II. All of the enteric-coated tablets resisted disintegration in gastric fluid after 1 h of testing. Cellulose acetate phthalate coating was thicker than polyvinyl acetate phthalate for the same number of coats.

The condition of the coated tablets, as indicated by the alphabetical rating (Table II), varied with the polymer used for coating and the number of coats. Thirty coats of the polyvinyl acetate phthalate formulation were required to provide complete protection to the tablet. Only seven coats of the cellulose acetate phthalate formulation were required. The tablets with five coats of cellulose acetate phthalate showed some signs of coat softening after 1 h in the gastric fluid. Tablet core disintegration, however, did not occur in this group after testing for 4 h in the gastric fluid. All batches had an average disintegration time in intestinal fluid of ≤22 min. There appeared to be no relationship between the number of coats and disintegration times.

The following criteria were established to help in the selection of the best coating material and the ideal number of coats based on dissolution testing:

1. Less than 10% of the drug should be released after 4 h at pH 1.2.
2. The enteric coat should not alter dissolution of the tablet at pH 6.0. There should be no significant difference in percent remaining in the uncoated or enteric-coated tablets at selected sampling times.
3. The enteric coat should not alter dissolution of the tablet at pH 8.0. There should be no significant difference in percent remaining in the uncoated or enteric-coated tablets at selected sampling times.

The dissolution profiles for the uncoated tablets and five batches of enteric-coated tablets at pH 1.2 are given in Table III. All of the coated formulations, except for polyvinyl acetate phthalate with 25 coats, retained ≥90% of the drug in the dosage form after 4 h of dissolution testing at pH 1.2. Less than 40% of the drug remained in the uncoated tablets after 30 min of testing.

A *t* test ($p = 0.001$) was used to determine whether criteria 2 and 3 were met by any of the formulations. The results of the analysis for pH 6.0 and pH 8.0 are given in Tables IV and V, respectively. Differences between the groups were more evident with the pH 6.0 buffer. Only tablets coated with five layers of cellulose acetate phthalate showed no difference at each sampling time when compared with the uncoated tablet group. By using the pH 8.0 buffer, only the 5-min sample of tablets coated with 30 coats of polyvinyl acetate phthalate differed from the uncoated group.

Table VI—Mean Disintegration Times for Enteric-Coated and Uncoated Tablets in Intestinal Fluid After Storage [a]

Temperature, °C	Mean Disintegration Time, min					
	0 d	15 d	30 d	45 d	60 d	75 d
	Enteric-Coated Tablets					
4	21	—[b]	21	—	21	20
25	21	—	20	—	21	21
37	21	—	22	21	22	20
50	21	29	20	20	17	18
	Uncoated Tablets					
4	21	—	15	—	18	20
25	21	—	19	—	25	25
37	21	—	19	19	20	20
50	21	17	16	19	17	17

[a] $n = 3$. [b] No sample taken.

[7] Model 4200 aerograph; Varian Associates.
[8] Partisil-10-SCXR; Whatman, Inc.
[9] LC-4A amperometric controller; Bioanalytical Systems, Inc.
[10] LC-19 flow cell; Bioanalytical Systems, Inc.
[11] Hemoccult slides; Smith Kline Diagnostics.

Table VII—Relationship Between Animal Weight and t_{max}

Animal	Weight, kg	C_{max}, μg/mL	t_{max}, h
C26	24.0	10.18	2.0
B23	29.0	16.46	1.5
C28	19.8	12.55	4.0
B24	18.5	11.84	3.0

Selection of the Best Coat—In general, the formulation containing cellulose acetate phthalate was more suited to the pan coating procedure than was the formulation which contained polyvinyl acetate phthalate. The tablets coated with cellulose acetate phthalate did not stick together and required fewer applications. Tablets coated with 5 layers of this formulation were judged to be better than tablets coated with 7 or 10 coats. Five applications prevented disintegration after 4 h of testing in simulated gastric fluid, although some damage occurred after 1 h. This batch of tablets was the only one to meet the specifications set for the dissolution study. The addition of two more layers improved the performance in the disintegration test but drastically altered dissolution at pH 6.0.

Stability of the Coat—Disintegration and dissolution tests were used to determine whether storage at different temperatures altered the release of drug from uncoated and enteric-coated penicillamine tablets. All enteric-coated tablets resisted disintegration in simulated gastric fluid after 1 h of testing. The degree of coat damage during the disintegration test varied from no obvious damage to some softening of the coat. There was no relationship between temperature or storage time and the amount of coat damage.

Storage had no effect on the disintegration time of enteric-coated tablets in intestinal fluid (Table VI). These results compare favorably with those reported previously by Luce (9). In that study, tablets coated with cellulose acetate phthalate showed no change in disintegration time after storage at room temperature for 1 year.

No changes in dissolution of the uncoated or enteric-coated tablets was seen at pH 1.2. Changes did occur in the dissolution of both the coated and uncoated tablets at pH 6. As shown by a typical profile (Fig. 1), the changes in dissolution were random. No relationship could be established between storage time or temperature. The apparent changes in dissolution could not be attributed to the presence of the enteric coat.

Plasma Concentrations—The plasma concentration-time curves for four animals are shown in Fig. 2. Data from one animal in each group were not included because the samples were not assayed until 10 d after collection, and degradation of the samples was suspected.

The curves show atypical double peaks. Similarly shaped curves have been reported after oral administration of penicillamine in a mongrel dog (10) and in normal human subjects (3). The multiple peaks did not occur after intravenous administration of the drug in the dog (10) or when given with food in humans (3). Double peaks after oral administration of cimetidine (11) and phenytoin (12) have also been reported in the literature. Explanations for these types of curves have included a GI transit-time effect (3) and an enterohepatic cyclic-process effect (11).

Examination of the plasma concentration-time curves (Fig. 2) reveals two types of curves, which differ in the size and location of the double peaks. The curve shape was independent of the type of tablet administered. Although the number of subjects was low, it appears that the shape may be related to the weight of the animal. In Table VII, the relationship between animal weight and the time (t_{max}) to reach the maximum plasma concentration (C_{max}) is shown. The lag time seen in the group receiving the enteric-coated tablet group was ~1 h.

The same two types of plasma concentration-time curves have been observed by Bergstrom *et al.* (3) and appear to be related to the weight of the subject. The areas under the plasma concentration-time curves (AUC) from 0 to 6 h were calculated by the trapezoidal rule. The average AUC from pigs that received enteric-coated tablets was compared with the average AUC from pigs that received uncoated tablets. Since the drug was not given on a milligram per kilogram basis, each AUC was normalized to a body weight of 24.0 kg. Both the calculated and normalized AUC values are given in Table VIII.

The mean, normalized, AUC for animals that received uncoated tablets was 27.61 mg·h/mL, as compared with 18.36 μg·h/mL for the animals that received enteric-coated tablets. The relative bioavailability of the enteric-coated tablet as compared with the uncoated tablet was 66.5%. The overall difference in AUC between the two groups was due primarily to the large difference between animals C26 and B23. A study with a larger number of subjects and a crossover design would be necessary to determine whether the bioavailability is reduced by the enteric formulation.

Table VIII—AUC from Pigs Receiving Either Uncoated or Enteric-Coated Penicillamine Tablets

Animal	AUC, μg·h/mL	Weight, kg	Normalized AUC, μg·h/mL
B23	26.95	29.0	32.56
B24	29.40	18.5	22.67
C26	16.15	24.0	16.15
C28	24.93	19.8	20.57

Occult Blood Tests—Stool specimens from each group of pigs were checked for occult blood daily for 55 d. The incidence of positive tests in the control group was 13%. Pigs receiving uncoated penicillamine tablets had positive tests on 44% of the days. The group receiving enteric-coated penicillamine tablets had 24% positive tests. An outbreak of vibrionic dysentery during the middle of the study was probably responsible for some of the positive tests. The *Vibrio coli* organism can cause bleeding from the GI tract (13). If the 21 d in which the animals were treated for dysentery are omitted, the percentage of positive tests for the control group, animals receiving uncoated tablets, and animals receiving enteric-coated tablets become 5.8, 35.3, and 11.8%, respectively. The incidence of blood in the stool of the group receiving uncoated tablets was significantly higher using either set of data. It appears, therefore, that less GI bleeding occurred in the pigs which were given enteric-coated penicillamine tablets than those given the uncoated tablets.

Tissue Analysis—No significant mucosal damage was noted in any of the groups.

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