Table V—Effects of Compounds 7 and 8, in which Sorbitan Fatty Acid Esters were Added, on Microorganism Growth

Sorbitan Ester	MIC, μg/ml			
	Streptococcus aureus		*Aspergillus niger*	
	Compound 7	Compound 8	Compound 7	Compound 8
None (control)	250	250	7.8	15.6
Monolaurate	<7.8	<7.8	7.8	15.6
Monopalmitate	125	125	7.8	15.6
Monostearate	250	125	7.8	15.6
Tristearate	62.5	125	15.6	15.6
Monooleate	125	250	7.8	15.6
Trioleate	62.5	125	7.8	31.3

surfactants were employed as solubilizing agents.

Table V shows the MIC of surfactants plus Compounds 7 and 8. The surfactants used had no antimicrobial activity. Only for *Streptococcus aureus* were the compounds with added surfactants more effective than those compounds alone. The sorbitan monolaurate suspensions of the compounds had good MIC values, below 7.8 μg/ml. Since the MIC's of Compounds 7 and 8 alone were 250 μg/ml, the compounds with the sorbitan fatty acid esters may have enhanced activities.

EXPERIMENTAL

***N*-(*N*-Substituted-thiocarbamoyl)cysteines**—These compounds were obtained by the method of Todrick and Walker (7).

2,3-Bis(substituted-thiocarbamoylthio)-1-propanols—2,3-Dimercapto-1-propanol was dissolved in about 30 parts of water. Two equimolar quantities of the corresponding isothiocyanate were added and gentle shaking was maintained for 3 hr, whereupon a yellow granular solid started to separate. The mixture was stored overnight and filtered, and the solid was recrystallized from ethanol.

In the preparation of 2,3-bis[aryl(aralkyl)-thiocarbamoylthio]-1-propanol, the same procedure was employed except that dimethyl sulfoxide was used instead of water.

Antimicrobial Activity—The antimicrobial activity was determined by determining the MIC (micrograms per milliliter) macroscopically or microscopically using the normal dilution method.

The concentration of each surfactant was 1 mg/ml of liquid medium (pH 6.0).

REFERENCES

(1) M. Kojima and K. Kobayashi, *J. Ferment. Technol.*, **41**, 508(1963).

(2) A. R. Saghir, L. K. Mann, R. A. Bernhard, and J. V. Jacobsen, *Proc. Amer. Soc. Hort. Sci.*, **84**, 386(1964).

(3) T. Zsolnai, *Arzneim.-Forsch.*, **16**, 870(1966).

(4) L. Drobnica, M. Zemanova, P. Nemec, K. Antos, P. Kristian, A. Stullerrova, V. Knoppova, and P. Nemec, Jr., *Appl. Microbiol.*, **15**, 701(1967).

(5) A. K. S. Jpesteyn and G. J. M. van der Kerk, *Biochim. Biophys. Acta*, **13**, 545(1954).

(6) A. F. McKay, D. L. Garmaise, R. Gaudry, H. A. Baker, G. Y. Paris, R. W. Kay, G. E. Just, and R. Schwartz, *J. Amer. Chem. Soc.*, **81**, 4328(1959).

(7) A. Todrick and E. Walker, *Biochem. J.*, **31**, 297(1937).

(8) O. Mumm and H. Richter, *Chem. Ber.*, **73**, 843(1940).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 16, 1973, from the *Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma, Nishi-ku, Fukuoka 814, Japan.*

Accepted for publication June 11, 1974.

The author thanks Dr. T. Yamano, Research Laboratory, Takeda Pharmaceutical Industries Ltd., for the antimicrobial activity experiments, and the members of the Analytical Center, Shizuoka College of Pharmacy, for the elemental analyses.

Transport Mechanisms of β-Lactam Antibiotics across Everted Rat Gut

STANLEY C. PENZOTTI, Jr.[x], and JOHN W. POOLE

Abstract □ Penicillins and cephalosporins, both monobasic and amphoteric, were tested for active transfer by measurement of the ratio of drug between serosal and mucosal solutions in a standard everted rat gut preparation. In no instance did any of these β-lactam antibiotics demonstrate active transport. Parallel experiments with glucose, a compound known to be actively transported in this preparation, always resulted in a positive ratio. A linear relationship between rate of transfer and concentration for two compounds, sodium ampicillin and sodium nafcillin, provided further evidence for passive transport. Although sodium nafcillin showed an apparent saturable process between 20 and 40 mg/ml, surface tension measurements showed that the CMC for sodium nafcillin lies within this range.

Keyphrases □ Antibiotics, β-lactam—transport mechanisms across everted rat gut □ β-Lactam antibiotics—transport mechanisms across everted rat gut □ Cephalosporins—transport mechanisms across everted rat gut □ Transport mechanisms—β-lactam antibiotics across everted rat gut

Since penicillins are known to be actively secreted into the renal tubules (1–3) and actively transported from the cerebrospinal fluid to the blood (4), the possibility that there may also be a specialized process in the intestinal absorption of β-lactam antibiotics must be considered. Earlier *in vitro* studies with an everted gut preparation indicated that the transport of phenoxymethyl penicillin and phenoxybenzyl penicillin (phenbenicillin) is a saturable process (5, 6), although additional studies did not produce evidence of active transport (6).

In the present work, the everted rat intestinal sac was employed to investigate the transport mechanism of several penicillins and cephalosporins, in-

Table I—Final Serosal-to-Mucosal Concentration Ratio across the Everted Rat Intestinal Sac, pH 7.4 Krebs–Henseleit Buffer, 37°

β-Lactam, 2 mg/ml	Serosal-to-Mucosal Concentration Ratio[a]					
	Nonsaturated			Saturated		
	20 min	40 min	60 min	20 min	40 min	60 min
Potassium penicillin G	0.93	0.87	0.74	0.92	0.81	0.69
Potassium phenoxymethyl penicillin	0.93	0.86	0.84	0.87	0.85	0.70
Sodium ampicillin	0.89	0.83	0.70	0.87	0.82	0.80
Sodium cyclacillin	0.91	0.89	0.91	0.92	0.88	0.86
Sodium nafcillin	0.89	0.88	0.69	0.85	0.77	0.63
Sodium dicloxacillin	0.84	0.74	0.60	0.94	0.81	0.79
Pivampicillin	0.95	0.84	0.84	0.90	0.71	0.71
Cephalexin monohydrate	0.93	0.75	0.72	1.05	0.89	0.83
Cephaloglycin dihydrate	0.61	0.57	0.50	0.72	0.64	0.76
Cephaloridine	0.92	0.88	0.93	1.00	1.04	0.73
Sodium cephalothin	0.87	0.87	0.64	0.84	0.75	0.64
Glucose[b], 1.8 mg/ml	1.33 ±0.19	1.73 ±0.39	2.43 ±0.76	1.44 ±0.20	1.84 ±0.36	2.54 ±0.71

[a] Average of duplicate sacs. [b] Average of 22 sacs ± *SD*.

cluding sodium ampicillin, sodium cyclacillin, cephalexin, and cephaloglycin. These four compounds are amphoteric β-lactams, exhibiting many physical–chemical properties common to amino acids (7) which are known to be actively taken up from the intestine (8).

EXPERIMENTAL

A large section of small intestine, starting approximately 15 cm below the pyloric sphincter, was removed from an ether-anesthetized rat, rinsed with iced saline, and everted. Three 10-cm sections were used from each of two animals. Each section was tied at one end, filled with 2 ml of pH 7.4 Krebs–Henseleit bicarbonate buffer (9) containing the drug at 2 mg/ml and 10 m*M* glucose, and then tied at the other end to form a sac. The sacs were suspended in a large test tube containing 100 ml of the same drug and glucose-containing buffer solution; *i.e.*, equal concentrations were established on both sides of the membrane. There were two sacs per tube, and each tube was continually gassed with 5% carbon dioxide in oxygen and maintained at 37°.

At specified intervals, the sacs were removed and the outsides were rinsed with saline and blotted dry. The inside (serosal) contents were removed and assayed for drug and glucose content. At the same time, a sample from the outside solution (mucosal side) was withdrawn for assay. A similar set of experiments was conducted with sacs that had been incubated previously in an identical aerated drug solution for 1 hr and thus saturated with drug on both the mucosal and serosal sides. These tissues were designated as treated segments.

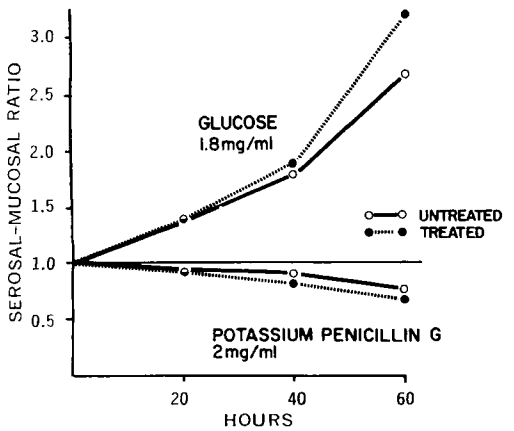

Figure 1—*Serosal-to-mucosal concentration ratios for glucose and potassium penicillin G in everted rat intestine after three periods of incubation (20, 40, and 60 min). Both drug-saturated (treated) and unsaturated (untreated) readings are shown. Each point represents the average of two preparations.*

The final serosal-to-mucosal concentration ratios were determined, and a ratio of greater than 1 was used as an indication of active transport. All determinations were done in duplicate. The 20- and 60-min incubations were carried out on one sac from the distal portion and one sac from the proximal portion of the intestine, and the 40-min incubation was done on the two middle sections. At no time was there any detectable difference in the behavior of segments from different portions of the intestine.

Two compounds, sodium ampicillin and sodium nafcillin, were selected for additional study to determine if the transport rate is proportional to the initial concentration. Glucose, a model compound for active transport (10), was included with each antibiotic tested to demonstrate the metabolic viability of the preparation. In these experiments the everted sacs, 15 cm in length, were filled with 3 ml of drug-free Krebs bicarbonate buffer and incubated in 100 ml of buffer containing the drug at the desired concentration. The transfer of the drug was followed with time, and the mucosal and serosal drug contents were measured as before at the end of 20 or 30 min.

In all cases the β-lactam antibiotics were assayed using the iodometric titration procedure (11). Glucose was determined enzymatically[1], and surface tension measurements were made with a surface tensiometer[2] at room temperature in the same buffer.

The penicillins studied[3] were potassium penicillin G, potassium phenoxymethyl penicillin (potassium penicillin V), sodium ampicillin, sodium cyclacillin, sodium dicloxacillin, sodium nafcillin, and pivampicillin. The cephalosporins[4] were sodium cephalothin, cephaloridine, cephaloglycin dihydrate, and cephalexin monohydrate. All other chemicals were reagent grade.

RESULTS AND DISCUSSION

Figure 1 shows the results for glucose and for potassium penicillin G, a typical monobasic penicillin, when the ratios of serosal-to-mucosal concentrations were examined at 20, 40, and 60 min. With both the treated and untreated preparations for glucose, there was a continuous accumulation of drug on the serosal side. With potassium penicillin G, however, no accumulation against a concentration gradient occurred at any of the three periods studied. In this case, and in fact for each drug studied, there was a slight decrease in the ratio with time, and the value was almost always less than 1. This result was due to a decrease in concentration on the inside or serosal side of the membrane and not to any increase on the mucosal side. The decrease was originally thought to be due to drug

[1] Using the Glucostat reagent, Glucostat TM, Worthington Biochemical Corp., Freehold, N.J.
[2] Fisher Surface Tensiomat, Fisher Scientific Co., Pittsburgh, Pa.
[3] Wyeth Laboratories, Radnor, Pa.
[4] Eli Lilly and Co., Indianapolis, Ind.

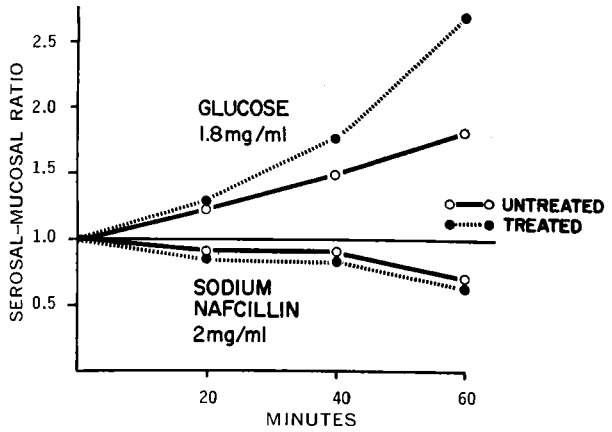

Figure 2—*Serosal-to-mucosal concentration ratios for glucose and sodium nafcillin in everted rat intestine after three periods of incubation (20, 40, and 60 min). Both drug-saturated (treated) and unsaturated (untreated) readings are shown. Each point represents the average of two preparations.*

binding to the tissue surface which, because of the smaller volume on the serosal side, might have a significant effect on the final concentration. However, as can be seen from the dashed lines (Fig. 1), there was also a similar decrease in the treated (saturated) preparations. Moreover, glucose behaved the same way regardless of treatment.

Figure 2 illustrates the results for a penicillinase-resistant compound, sodium nafcillin. The behavior of this drug was similar to that of potassium penicillin G; *i.e.*, a decrease in the ratio with time occurred with both the treated and untreated segments. Again, glucose accumulated against a concentration gradient.

Table I summarizes the data on all of the compounds run. In all trials, glucose accumulated on the serosal side at 2–2.5 times its initial concentration. Control studies in the absence of any antibiotic gave similar results. Although the serosal-to-mucosal concentration ratios appear greater with the saturated segment, the unsaturated values compared to the saturated values at each time period were found not to be significantly different by the Student *t* test. The reason for the apparently higher ratios lies in the fact that the initial mucosal glucose concentrations were lower in the saturated segments due, most likely, to the metabolism of glucose by the unsaturated segments run previously. Although there was a corresponding decrease in the amount accumulated on the serosal side in the saturated guts, the decrease was proportionally less than the decrease in the mucosal concentration, so a slightly higher serosal-to-mucosal concentration ratio resulted.

The β-lactam antibiotics (except in three instances) exhibited ratios of less than 1, whether treated (saturated) or not. The three exceptions were the 20-min saturated value for cephalexin monohydrate and the 20- and 40-min saturated ratios for cephaloridine. Even in these cases, the values at the end of 1 hr were less than 1. The reason for the decrease is not known. Previous workers with

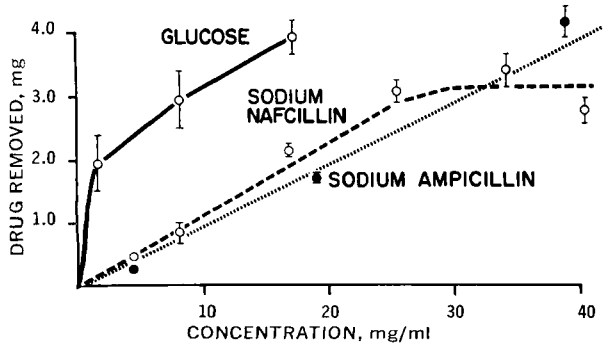

Figure 3—*Total amount of drug (milligrams) transferred across the everted rat intestine in 20 min (glucose and sodium ampicillin) or 30 min (sodium nafcillin) at various concentrations. Each point represents the average of at least two experiments, with the bars representing the range.*

Table II—Clearance Rates for Transport across Everted Rat Intestinal Sacs at Various Concentrations

Compound	Initial Concentration, mg/ml	Clearance Rate, ml/hr
Sodium ampicillin	1.0	0.26[a]
	4.2	0.27
	19.0	0.28
	38.7	0.32
Sodium nafcillin	1.0	0.21[a]
	4.2	0.23
	8.0	0.21
	16.8	0.26
	25.2	0.24
	34.3	0.20
	40.5	0.14
Glucose	1.4	4.04
	8.0	1.10
	17.0	0.69

[a] Studies run in the absence of glucose.

everted gut preparations have shown that in the presence of glucose there is a net transfer of water of between 0.1 and 0.3 ml/cm/hr from the mucosal to the serosal side (12). Therefore, if the transport of the antibiotic lags behind, a dilution could occur on the serosal side and could account for the lower values found.

Further evidence for passive transport was provided by studies of the effect of increasing concentration on transfer rate. Figure 3 shows the amount transferred in 20 min for sodium ampicillin and glucose and in 30 min for sodium nafcillin. Glucose gave the expected result for an active transport process since the amount removed did not increase proportionately with the increase in concentration. Data for sodium ampicillin were typical for a compound that is passively transported; *i.e.*, there was a linear response to the increasing concentration. Results for sodium nafcillin were indicative of something more than the simple passive transport suggested by previous data for this compound. Although there was an apparent linear increase in the amount transferred up to approximately 25–30 mg/ml, a leveling of the rate was observed above this concentration.

These data can be expressed in another fashion, *i.e.*, as clearance rates (Table II). The clearance rate was calculated by dividing the amount transferred in a unit time (here normalized to 1 hr) by the initial concentration. A constant clearance rate indicates passive transport. As can be seen, the values for glucose fell off dramatically, decreasing from approximately 4 ml/hr at 1.4 mg/ml to 0.7 ml/hr at the highest concentration (17 mg/ml), thus indicating active transport. The rates for sodium ampicillin remained constant over a 10-fold change in concentration. For sodium nafcillin the values were essentially constant up to 25–30 mg/ml but started decreasing above this concentration. Control studies for both antibiotics run in the absence of glucose demonstrated that glucose did not affect their transport.

If it is assumed from the sodium nafcillin data that no active process is occurring at the lower concentrations, then some change

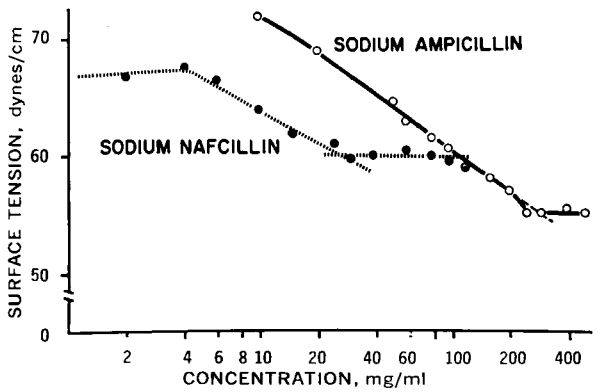

Figure 4—*Surface tension (dynes per centimeter) of sodium ampicillin and sodium nafcillin at various concentrations.*

in the form of the drug must be taking place at the higher concentrations. Evidence in the literature indicates that a variety of surface-active compounds may undergo association (13), and when such association takes place the monomer concentration can no longer be equated to stoichiometric concentrations. Certain β-lactam antibiotics have shown surface-active properties (14).

Figure 4 shows a plot of log concentration *versus* surface tension for the sodium salts of nafcillin and ampicillin. The apparent critical micelle concentration (CMC) for sodium nafcillin (the break in the plot) is in the range of 20–30 mg/ml, the same point where saturation of the transport was observed. Sodium ampicillin, on the other hand, has a CMC above the concentrations used in the transport studies.

In summary, these studies show that the β-lactam antibiotics (both penicillins and cephalosporins) are not transported across the everted rat gut by any specialized transport mechanism. This was true for both penicillinase-sensitive and penicillinase-resistant compounds in this series, as well as for compounds (amphoteric) resembling amino acids. In addition, the results obtained with the model compound, glucose, demonstrate the utility of the everted rat gut for this type of investigation.

REFERENCES

(1) A. Despopoulos, *J. Theoret. Biol.*, **8**, 163(1965).

(2) P. Acred, D. M. Brown, D. H. Turner, and D. Wright, *Brit. J. Pharmacol. Chemother.*, **17**, 70(1961).

(3) P. Acred, D. M. Brown, D. H. Turner, and M. J. Wilson, *ibid.*, **18**, 356(1962).

(4) R. L. Dixon, E. S. Owens, and D. P. Rall, *J. Pharm. Sci.*, **58**, 1106(1969).

(5) I. M. Rollo, *Pharmacologist*, **6**, 177(1964).

(6) I. M. Rollo, *Can. J. Physiol. Pharmacol.*, **50**, 986(1972).

(7) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, **58**, 1510(1969).

(8) G. Wiseman, *J. Physiol.*, **120**, 63(1953).

(9) H. A. Krebs and K. Henseleit, *Hoppe-Seyler's Z. Physiol. Chem.*, **210**, 33(1932).

(10) R. B. Fisher and D. S. Parsons, *J. Physiol. (London)*, **110**, 281(1949).

(11) J. W. Poole and C. K. Bahal, *J. Pharm. Sci.*, **57**, 1945(1968).

(12) R. B. Fisher and D. S. Parsons, *J. Physiol. (London)*, **110**, 36(1949).

(13) P. Mukerjee, *Advan. Colloid Interface Sci.*, **1**, 241(1967).

(14) E. D. Purich, J. L. Colaizzi, and R. I. Poust, *J. Pharm. Sci.*, **62**, 545(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 27, 1974, from the *Exploratory Pharmacy Section, Pharmacy Research and Development Division, Wyeth Laboratories, Inc., Radnor, PA 19087*

Accepted for publication June 11, 1974.

The authors thank Dr. James Boylan of Eli Lilly and Co. for kindly supplying cephalexin monohydrate and cephaloglycin dihydrate, and Paul Osimo for technical assistance.

[x] To whom inquiries should be directed.

New Carcinostatic Agent with Possible Selective Activity on Tumor Cells

I. NABIH [x] and A. ZAYED

Abstract □ The nitrogen mustard analog of the antitumor agent 1,2-cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone was synthesized. Its biological activity in experimental tumors *in vivo* and on human leukemia *in vitro* is described.

Keyphrases □ Nitrogen mustard analog of 1,2-cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone—synthesis and screening for carcinostatic and antitumor activities □ 1,2-Cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone, nitrogen mustard analog—synthesized and screened for carcinostatic and antitumor activities □ Antitumor activity—synthesis and screening of nitrogen mustard analog of 1,2-cyclohexeno-4-(β-diethylaminoethylamino)-thiaxanthone

Previous articles (1, 2) reported the antitumor activity of a new group of compounds related to the cyclohexenothiaxanthones. These are mainly represented by the three isomers 1,2-cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone (I), 2-(β-diethylaminoethylamino)-3,4-cyclohexenothiaxanthone (II), and 1-(β-diethylaminoethylamino)-3,4-cyclohexenothiaxanthone (III).

DISCUSSION

Structurally, the cyclohexenothiaxanthone system incorporates the thiaxanthone skeleton along with that of tetrahydronaphthalene. The rational for this combination is that the thiaxanthone tends to polarization due to resonance (3), thus indicating separation of charges and high dipole moments. This physical property would permit a selective attack on the tumor cells, avoiding the normal ones, at the appropriate concentrations, since the cellular membranes of the former carry considerably higher negative charges than their normal homologs (4, 5). Studies indicate that the thiaxanthone moiety in these compounds could be used as a carrier or a target-seeking device for the selective attack on tumor cells through electrostatic attraction.

The other structural part of the molecule, the tetralin, tends to attach molecular oxygen in an oxygenation process (6). Such oxygen carriers could affect the glycolysis in the cell by oxidation of the essential SH-groups of some enzymes necessary for cell growth and replication. In addition, the tetralins could undergo biological transformation to naphthoquinone-type structures, which are known to depress cell division.

In experimental tumors of the Ehrlich ascites carcinoma type, mammary carcinoma, Maloney virus lymphoma, and mouse ascites leukemia, the three isomers showed pronounced antitumor effects (1, 2). *In vitro* experiments on cells of these tumor types, as well as on leucocytes from several forms of human leukemia, showed that the compounds possessed varying but definite damaging effects on malignant cells.

The present work illustrates the synthesis of an alkylating agent with properties similar to those of nitrogen mustards, where the cyclohexenothiaxanthone structure may retain its antitumor effect and also serve as a carrier for the alkylating group. This is of importance in relation to the transport and chemical reactivity of this group. This approach had received considerable interest con-