Table III—Effect of Compounds I–X on Gastric Secretion in the Pylorus-Ligated Rat

	No. of Rats	Dose mg/kg p.o.	Volume of Gastric Secretions: ml/100 g Body Wt.	Volume of Gastric Secretions: Mean Percent of Control[b]	Total Gastric Acid Output: meq/100 g Body Wt.	Total Gastric Acid Output: Mean Percent of Control[b]	ID_{50}[c], mg/kg p.o. (95% Confidence Limits)
Control[a]	5		3.01 ± 0.79		0.18 ± 0.059		
I	3	50	1.99 ± 0.33	66	0.096 ± 0.028	53	5.7 (1.0–21.0)
Control[a]	5		3.44 ± 0.89		0.23 ± 0.076		
II	3	100	2.30 ± 0.50	67	0.097 ± 0.035	42	22.1 (11.7–37.0)
Control[a]	5		4.65 ± 0.71		0.31 ± 0.066		
III	3	100	1.78 ± 0.20	38	0.063 ± 0.0050	20	26.7 (19.2–31.0)
Control[a]	5		3.46 ± 0.60		0.21 ± 0.060		
IV	3	100	2.24 ± 0.63	65	0.12 ± 0.041	57	24.7 (19.2–31.0)
Control[a]	5		1.85 ± 0.62		0.095 ± 0.052		
V		100	0.83 ± 0.12	45	0.057 ± 0.012	60	25.9 (11.9–50.7)
Control[a]	5		5.00 ± 0.76		0.31 ± 0.083		
VI		100	4.07 ± 1.16	81	0.37 ± 0.12	121	—
Control[a]	5		5.00 ± 1.27		0.31 ± 0.083		
VII	3	100	1.78 ± 0.58	36	0.14 ± 0.064	45	>180
Control[a]	5		5.62 ± 0.18		0.61 ± 0.049		
VIII	3	100	1.34 ± 0.21	24	0.062 ± 0.010	10	57.4 (51.6–64.9)
IX	3	100	1.48 ± 0.29	26	0.073 ± 0.017	12	43.4 (24.4–111.3)
X	3	100	2.22 ± 0.055	39	0.014 ± 0.091	23	50.4 (30.1–127.1)

[a] The control values were obtained by peroral administration of 0.50% methylcellulose solution to a group of rats at 1 ml/100 g % body weight. [b] Compared to control (nondrug-treated) reading 4 hr after pylorus-ligation of rat stomach. [c] The dose eliciting 50% inhibition of total gastric acid output; calculated from data obtained at doses of 10, 30, and 100 mg/kg p.o., by method of linear regression analysis (5) of the dose–response curve.

ligation. Under light ether anesthesia, the rat stomach was ligated at the pylorus region. The conscious rat was sacrificed by a chloroform overdose 4 hr after ligation. The stomach was carefully excised and its contents drained into a centrifuge tube. Samples were centrifuged to separate secretions from debris, and gastric fluid volume was read and recorded. Titration was performed on a sample aliquot of 1 ml diluted to a volume of 5 ml using distilled water. The titrant used was 0.1 *N* NaOH. Acid concentration in the stomach was determined by titration to pH 7. Total gastric acid output was calculated as the product of volume of gastric secretions and acid concentration for each compound tested.

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Effect of Nutrient Depletion on the Sensitivity of *Pseudomonas cepacia* to Antimicrobial Agents

R. M. COZENS * **and M. R. W. BROWN** ˣ

Received June 28, 1982, from the *Microbiology Research Group, Department of Pharmacy, University of Aston in Birmingham, Birmingham B4 7ET, England.* Accepted for publication October 7, 1982.

* Present address: CIBA-GEIGY Ltd., Infectious Diseases Research, Basle, CH-4002 Switzerland.

Abstract □ *Pseudomonas cepacia* depleted of various nutrients showed marked variation in sensitivity to cetrimide, chlorhexidine, and benzalkonium chloride. In all cases cells depleted of magnesium were the most resistant. It is proposed that these observations may be due to alterations of the envelope of *P. cepacia* in response to changes in the growth environment. This may have profound implications for investigations of the resistance of this organism both *in vivo* and *in vitro*.

Keyphrases □ Nutrient depletion—effect of senstivity of *Pseudomonas cepacia* to antimicrobial agents □ Antimicrobial agents—effect of nutrient depletion on sensitivity of *Pseudomonas cepacia* □ *Pseudomonas cepacia*—effect of nutrient depletion on sensitivity to antimicrobial agents

Pseudomonas cepacia, previously considered as only a plant pathogen, has, in the last decade, been implicated in nosocomial infections with increasing frequency (1–3). The organism is more resistant to most useful antimicrobial agents than other Gram-negative bacteria. It is capable of survival and even multiplication in quaternary ammonium compounds (2, 4, 5) and will multiply in distilled or deionized water (6, 7).

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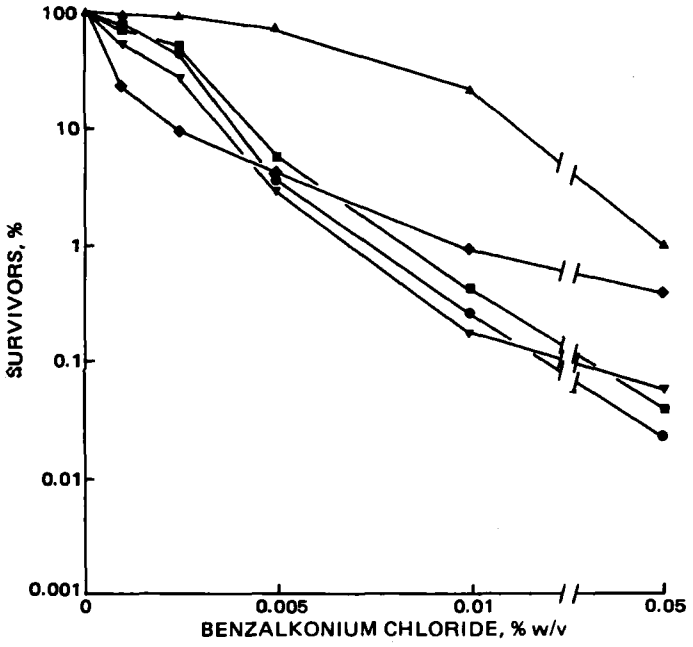

Figure 1—*The effect of benzalkonium chloride on cells of* P. cepacia *depleted of glucose (●), magnesium (▲), and ammonium (■), phosphate (◆), and ferrous ions (▼).*

The relationship between susceptibility to antimicrobial agents and the growth environment has been recognized for some time (8). Brown (9) has emphasized the need for the use of nutritionally defined cultures for the investigation of the response of bacteria to antimicrobial agents and host defense mechanisms. As *P. cepacia* in its natural environment or as a contaminant of disinfectant solutions is probably depleted of an essential nutrient (9), the use of cultures depleted in this way would possibly be more relevant in investigations of the extreme resistance of this organism. This paper describes the effect of nutrient depletion on the resistance of *P. cepacia* to some common antimicrobial agents.

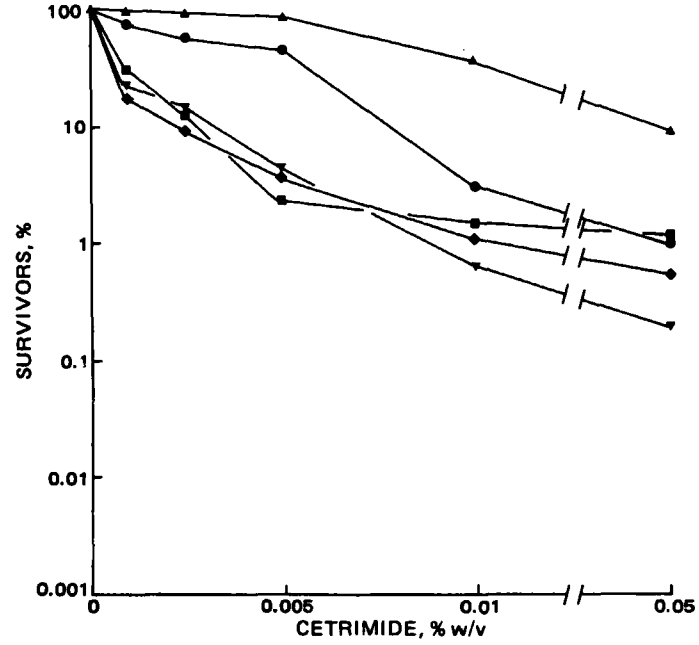

Figure 2—*The effect of cetrimide on cells of* P. cepacia *depleted of various nutrients (see Fig. 1 for key).*

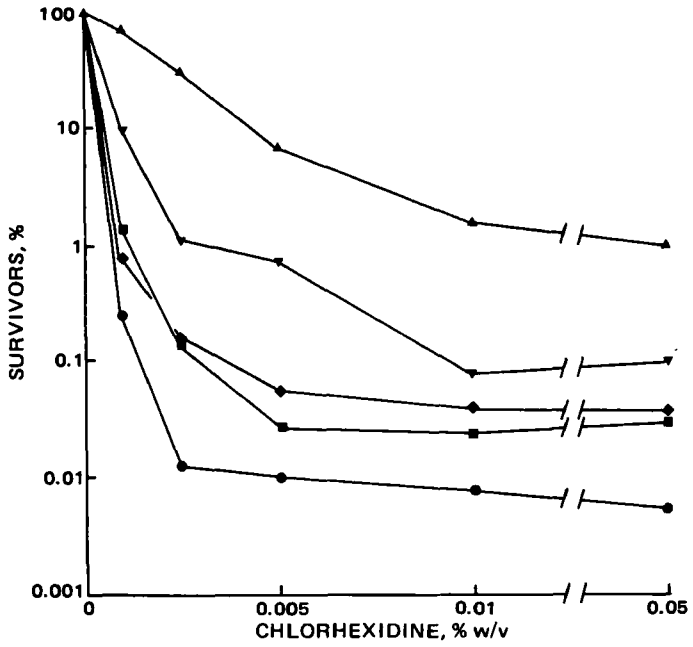

Figure 3—*The effect of chlorhexidine on cells of* P. cepacia *depleted of various nutrients (see Fig. 1 for key).*

EXPERIMENTAL

Organism—*Pseudomonas cepacia* NCTC 10661 was used throughout. Cultures were maintained by monthly subculture on nutrient agar slopes stored at 4°. The basic medium contained 20 m*M* of glucose, 12 m*M* of $(NH_4)_2SO_4$, 1.2 m*M* of K_2HPO_4, 3.2 m*M* of $MgSO_4 \cdot 7H_2O$, 0.02 m*M* of $FeSO_4 \cdot 7H_2O$, 3 m*M* of NaCl, 3 m*M* of KCl, and 50 m*M* of 3-(*N*-morpholino)propanesulfonic acid (I), pH 7.4. Chemicals[1] were of the purest grade available.

For nutrient depletion studies the concentrations were separately adjusted: 2.0 m*M* of glucose, 0.5 m*M* of $(NH_4)_2SO_4$, 0.1 m*M* of K_2HPO_4, 0.01 m*M* of $MgSO_4 \cdot 7H_2O$, and no added iron to give the respective depletions. These concentrations allowed the cultures to reach maximum exponential phase at A_{470} = 0.5. Cultures (25 ml) were grown in baffled 100-ml Erlenmeyer flasks at 37° in a reciprocal shaking water bath at 120 throws/min.

Resistance to Antibacterial Agents—Cells grown under various nutrient depletions, as described above, were harvested by centrifugation, washed twice in saline buffered with I (pH 7.4) and finally resuspended to a A_{470} = 1.0. Cell suspension (0.1 ml) was added to 9.9 ml of various concentrations of drug (cetrimide[1], benzalkonium chloride[2], chlorhexidine gluconate[3]) prepared in saline buffered with (I). After 15 min at 37° samples were removed, the drugs inactivated by dilution in lecithin–polysorbate 80 broth, and suitable dilutions were incorporated into pour plates of nutrient agar. The plates were incubated for 36 hr at 37° before counting the resulting colonies.

RESULTS AND DISCUSSION

Figures 1–3 show the variation in sensitivity to antibacterial agents when the depleting nutrient was altered. In all cases, cells depleted of magnesium showed the greatest resistance to the action of these agents. Of the other depletions examined, the resistances against benzalkonium chloride and cetrimide showed little variation. Chlorhexidine showed a wider spread of activity, with glucose-depleted cells being the most sensitive.

Al-Hiti and Gilbert (10) have shown that those organisms specified for use in the USP XIX test for the effectiveness of antimicrobial preservatives exhibited a changed sensitivity to chlorhexidine diacetate and benzalkonium chloride, when depleted of different nutrients. The results described here show that *P. cepacia* exhibits similar changes in sensitivity to antimicrobial agents caused by different nutrient depletions.

[1] BDH Chemicals, Poole, UK. Listed in The British Pharmacopoeia as mainly tetradecyltrimethylammonium bromide with smaller amounts of dodecyl- and hexadecyltrimethylammonium bromide.
[2] Sigma, Poole, UK.
[3] ICI Ltd., Macclesfield, UK.

The cell envelope, and particularly the outer membrane, of Gram-negative bacteria is known to change in response to changes in the growth environment (8, 11, 12). It is therefore likely that these changes in resistance may reflect changes in the cell envelope which either prevent access of the drug to the site of action or alter the site of action such that the drugs show decreased activity. Little is known about the cell envelope of *P. cepacia*. The lipopolysaccharide is atypical, not containing 2-keto-3-deoxyoctonate and with several quantitative differences in the sugar moieties (13). The cellular fatty acid compositions (mostly derived from the envelope phospholipids) of *P. cepacia* and *Pseudomonas aeruginosa* differ in that the former has proportionately more cyclopropane fatty acids than does the latter (14).

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COMMUNICATIONS

Mean Hepatic Transit Time in the Determination of Mean Absorption Time

Keyphrases □ Mean hepatic transit time—determination of mean absorption time, pharmacokinetics □ Mean absorption time—determination by mean hepatic transit time, pharmacokinetics □ Pharmacokinetics—mean hepatic transit time, mean absorption time, mean residence time

To the Editor:

In recent studies the statistical moment theory has been employed to estimate mean absorption time (MAT) of drugs (1–5). The MAT has been defined as the mean time of a molecule (1) or the mean residence time (MRT) of all molecules (2) from a dosage form (such as solution or tablet) spent at the input site (GI lumen in the case of oral administration) before being absorbed into the general circulation. The MAT after oral administration has been calculated based on the following equation (1–5):

$$MAT = MRT_{po} - MRT_{iv} \qquad \text{(Eq. 1)}$$

where MRT_{po} is the MRT of the orally absorbed drug molecules in the body, and MRT_{iv} is the MRT of intravenously (usually from a peripheral vein in the leg or arm) administered drug molecules in the body. Both plasma and urinary excretion data have been proposed to estimate the MRT_{po} and MRT_{iv}. When a solution dosage form is studied, the calculated MAT has been referred to as mean intrinsic absorption time (5). When both solution and solid dosage forms are evaluated, the difference in their MRT may be considered to equal the mean *in vivo* dissolution time (MDT) from the solid dosage form. This is based on the assumption that once released from the solid dosage form, it is subject to the same influence as the drug administered in solution (1–3).

The main purpose of this communication is to discuss a complication in using Eq. 1 to determine the MAT. Its potential significance in absorption rate calculations and hepatic clearance studies will also be briefly mentioned. In analogy to the above assumption requirement in the determination of MDT, use of Eq. 1 must also require that the orally absorbed drug is handled in the body exactly the same way as that administered intravenously. This demand apparently can not be met because the orally absorbed drug has to pass through the GI wall and then the liver before entering the heart, while the intravenously administered drug can be carried almost instantaneously from the injection site to the heart before being distributed to the rest of body. The mean time to pass through the GI wall and liver can be called mean GI wall transit time, MTT_{GI}, and mean hepatic transit time, MTT_h, respectively. The portal circulation between the GI wall and liver is extremely fast and can be ignored. Therefore, Eq. 1 can be modified to:

$$MAT = MRT_{po} - MRT_{iv} - MTT_{GI} - MTT_h \qquad \text{(Eq. 2)}$$

The MTT in a tissue or organ during a single passage can be determined directly by instantaneous injection of a compound into the affluent blood and monitoring of effluent blood concentration, C_{out}, under the single-pass nonrecirculating condition (6–8):

$$MTT = \frac{\int_0^\infty tC_{out}dt}{\int_0^\infty C_{out}dt} \qquad \text{(Eq. 3)}$$

0022-3549/83/1100-1365$01.00/0