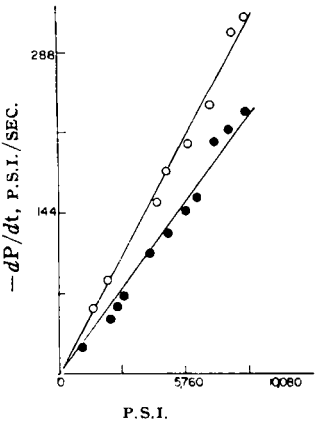

Fig. 5.—Effect of maximum pressure on initial relaxation slope for sodium chloride. Key: O, 1.8 seconds loading time; ●, 3.4 seconds loading time.

for the case $\Delta V/V$ constant. For a specific time period, a, the exponentials are constant and

$$\frac{-dp}{dt} = \sum_{i=1}^{n} \frac{e^{-a/\tau_i}}{\tau_i} P_i \qquad \text{(Eq. 3)}$$

Equation 3 reduces to

$$\frac{-dp}{dt} = k P \qquad \text{(Eq. 4)}$$

for all elements relaxing from an initial pressure P at fixed time. Accordingly, relaxation data for sodium chloride samples compressed at several pressures (weight and platen adjusted to keep $\Delta V/V$ constant) were obtained at two loading rates. Figure 5 is a plot of initial $-dp/dt$ against maximum pressure obtained at 1.8 seconds for 1.8-second loading time and 3.4 seconds for 3.4-second loading time.

DISCUSSION AND CONCLUSIONS

These preliminary studies were conducted to ascertain the feasibility of using stress relaxation data to investigate powder compaction. On the basis of the results obtained, the techniques appear to be valuable. Even though instrumentation was crude, evidence was obtained to show that factors known to affect tableting also had effects on stress relaxation data. The fact that seemingly anomalous behavior appears in some of these results is undoubtedly due to lack of experimental control or inadequate knowledge of the compression mechanism.

Certainly, correlation between tablet quality and bonding strength, surface area and porosity of the tablet, elastic compressibility, relaxation, particle size and shape, bulk density and flow properties of the powder may be expected. The technique reported in this communication is useful for studying elastic compressibility, relaxation, and flow under pressure. These important properties have received little attention in past studies on pharmaceutical powders; their measurement should help in understanding powder compression.

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Notes

Mode of Action of Endomycin-Neomycin Synergism

By W. T. SOKOLSKI and M. R. BURCH

The mode of action for endomycin-neomycin synergism against *Candida albicans* was postulated to be an action of endomycin which influences permeability of the cell membrane and facilitates the entry of neomycin in the cell. A neomycin-resistant strain was also found to be resistant to endomycin, but the combination of antibiotics was still synergistic. The permeability hypothesis of endomycin was strengthened by data showing that the cell membrane barrier was less resistant to increasing osmotic pressure in the presence of endomycin.

ENDOMYCIN is an antifungal and antibacterial complex of agents first described by Gottlieb, *et al.* (1). Endomycins A and B were reported to be synergistic with neomycin against *Candida albicans* UC1392 (2). Because endomycin lowers the surface tension of water and hence acts like a detergent, a cell wall permeability change has been postulated to be a factor in the synergism of endomycin and neomycin; presumably endomycin facilitates the passage of neomycin through the cell membrane. A precedent for this detergent type of synergistic activity was reported by Sokolski, *et al.* (3), and by Karaila (4).

One approach to show that endomycin affects the cell wall permeability was to use an organism which was resistant to neomycin. According to our hypothesis, the resistant organism should be sensi-

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TABLE I.—GROWTH OF *C. albicans* PARENTAL AND RESISTANT CULTURES IN THE PRESENCE OF NEOMYCIN, ENDOMYCIN, AND COMBINATIONS

C. albicans Strain	Antibiotic: Neomycin, mcg./ml.	Antibiotic: Endomycin, u./ml.	Absorbance, 650 mμ: Incubation, hrs. 20	44	120
Sensitive-parent	200	...	0.05	0.05	0.05
	50–100	...	0.05–0.06	0.05	0.25–0.33
	10–20	...	0.14–0.27	0.38–0.40	0.48–0.59
	5	...	0.42	0.47	0.71
	...	8–10	0.04	0.14–0.23	0.37–0.39
	...	1–4	0.23–0.34	0.37–0.39	0.38–0.45
	10	2	0.04	0.04	0.04
	5	2	0.04	0.04	0.27
	0	0	0.42	0.45	0.59
Neomycin-resistant	200	...	0.14	0.33	0.43
	50–100	...	0.23–0.34	0.47–0.51	0.52–0.61
	10–20	...	0.45	0.53–0.54	0.63–0.66
	5	...	0.50	0.53	0.63
	...	8–10	0.36–0.41	0.42–0.46	0.45–0.48
	...	1–4	0.42–0.43	0.48–0.51	0.52–0.54
	10	2	0.06	0.06	0.06
	5	2	0.06	0.06	0.06
	0	0	0.44	0.48	0.58
Endomycin-resistant	50–200	...	0.05	0.05	0.05–0.17
	10–20	...	0.05	0.04–0.40	0.39–0.60
	5	...	0.37	0.50	0.60
	...	10–20	0.13–0.39	0.25–0.41	0.29–0.60
	...	1–8	0.41–0.48	0.48	0.48–0.52
	5	2	0.03	0.03	0.04
	0	0	0.46	0.51	0.55

tive to neomycin in the presence of subinhibitory concentrations of endomycin. A second and possibly more convincing approach was to show that *C. albicans* was less resistant to increasing osmotic pressure in the presence of endomycin.

METHODS

The preparation of neomycin used in these studies was a commercial grade neomycin sulfate (Res. 9291) which was estimated (5) to contain 90% neomycin B and 10% neomycin C. Antibiotic potency was determined to be 650 mcg. neomycin B base equivalent per milligram. All concentrations used in these experiments were expressed in terms of neomycin B base equivalent.

The endomycin preparation was an amorphous sodium salt that was arbitrarily assigned a potency of 1000 units per milligram. The preparation was found to contain endomycins A and B, both of which were reported (2) as being synergistic with neomycin.

Neomycin-resistant and endomycin-resistant strains of *C. albicans* UC 1392 were developed by stepwise transfers to increasing concentrations of antibiotic in broth medium (yeast assay broth) consisting of 1% cerelose (glucose), 0.25% yeast extract, and 0.1% monopotassium phosphate. The neomycin-resistant strain was maintained in yeast assay broth containing 100 mcg. neomycin per milliliter, the endomycin-resistant strain in broth with 10 units endomycin per milliliter, and the parent strain in broth with no antibiotic.

The test organisms for inoculum in the determination for minimum inhibitory concentrations (MIC) were grown in shaken flasks with yeast assay broth and antibiotic for 24 hours at 37°. The cells from each culture were recovered by centrifugation, washed twice, and resuspended with the medium. The suspension was adjusted to an absorbance of 0.5 at 650 mμ.

The MIC tests were conducted in 18 × 150-mm. tubes which were optically matched for a Lumetron colorimeter. Sterile filtered antibiotic solutions were added to duplicate tubes which, when diluted to 10 ml., gave the concentrations indicated in Table I. The volumes in all tubes were brought to 2 ml. with sterile water. For each culture, 0.15 ml. of the inoculum suspension was added to 500 ml. of 10/8th strength yeast assay broth, after which 8 ml. of seeded broth were pipeted to each tube in the respective series. All tubes were momentarily agitated and then incubated in a 37° water bath.

Commercially available detergents were also tested for synergism with neomycin as was the combination of endomycin and detergents with potassium chloride. These tests were conducted against the parent *C. albicans* in the same manner as described above. Two anionic detergents, sodium lauryl sulfate and sodium heptadecyl sulfate, were also tested for synergism with neomycin. The concentrations used are indicated in Table II.

TABLE II.—SYNERGISM BETWEEN NEOMYCIN AND ANIONIC DETERGENTS AGAINST *C. albicans* PARENTAL

Detergent	Concn., %	Absorbance, 650 mμ[a]: Without neomycin	With neomycin[b]
Sodium lauryl sulfate (sodium dodecyl sulfate)	0.01	0.42	0.05
	0.005	0.40	0.05
Sodium heptadecyl sulfate	0.02	0.38	0.05
	0.01	0.41	0.05
None	...	0.45	0.48

[a] After 24 hours incubation at 32°C. [b] Ten micrograms neomycin B base equivalent.

RESULTS AND DISCUSSION

The results of the effect of endomycin, neomycin, and the combination against the parent and two antibiotic-resistant strains of *C. albicans* are shown in Table I. Absorbance at 650 mμ was used as an indication of the amount of growth. Microscopic wet-mount examinations were made of all tubes containing growth for the detection of bacterial contamination. No contaminated cultures were observed.

The response of the parent strain confirmed the synergistic activity with endomycin and neomycin previously reported (2). The results after 120 hours incubation show that 10 mcg. neomycin plus 2 units endomycin per milliliter inhibited growth and gave the same result as 200 mcg. neomycin per milliliter or >10 units endomycin per milliliter. Individually, 100 mcg. neomycin or 10 units endomycin per milliliter allowed some growth to occur.

The endomycin-resistant strain was more sensitive to neomycin than was the parent strain, as indicated by the lesser growth with neomycin alone at all reading periods. Synergism with the combination of antibiotics was apparent since no growth occurred with as little as 5 mcg. neomycin plus 2 units endomycin per milliliter, whereas growth was observed with 50 mcg. or units per milliliter, respectively.

The neomycin-resistant strain acquired resistance to endomycin since there was good growth with 10 units endomycin per milliliter after 20 hours. In the presence of the combination, however, synergism still existed. Five micrograms of neomycin plus 2 units per milliliter of endomycin was more effective in preventing growth than 500 units of neomycin or 10 units per milliliter of endomycin alone.

The fact that the neomycin-resistant strain was also resistant to endomycin may mean that the cell wall or membrane was altered during the development of resistance so that less antibiotic was absorbed and/or penetrated. The combination of the two agents was still synergistic, indicating that the action of endomycin was still effective in allowing more neomycin to enter the cell.

Table II shows that other anionic detergents were also synergistic with neomycin. These data are consistent with the concept that a chemical which can alter the permeability of the cell wall may be synergistic with antibiotics which are active in the cell. This detergent-antibiotic type of synergism has been shown to occur against the protozoa *Ochromonas danica* (3). A correlation between the surface tension-reducing activity on water and synergism with neomycin apparently was not present, but this is not surprising since the detergent action occurs between the cell and the surrounding liquid while the surface tension-depressing action is between the liquid and air.

Table III.—Influence of Endomycin on the Growth of *C. albicans* in Varying Concentrations of Potassium Chloride

Agents, Concn.		Absorbance, 650 mμ	
KCl, %	Endomycin, u./ml.	24 hr.	48 hr.
10	...	0.06	0.18
8	...	0.10	0.20
6	...	0.17	0.27
0.4 - 4.0	...	0.24–0.28	0.28
...	8	0.01	0.02
...	3–6	0.01–0.08	0.26–0.31
...	0.4–2	0.24–0.28	0.32
8	1	0	0.18
4–6	1	0.05 - 0.08	0.20–0.29
0.2 - 2	1	0.23 - 0.27	0.27–0.30
8	2	0	0
6	2	0	0.14
4	2	0.02	0.15
0.2 - 2	2	0.17–0.25	0.25–0.30
0	0	0.28	0.31

The possibility of a salt formation between neomycin and anionic detergents cannot be excluded. However, we previously could not show a third component with solutions containing neomycin and endomycin (3).

If the mode of action for endomycin-neomycin synergism is the action on the cell permeability by endomycin, then the cells should be less resistant to osmotic shock in the presence of endomycin; this was shown to be the case. Table III indicates that growth was inhibited with lower concentrations of potassium chloride in the presence of endomycin than without endomycin. Similar results were obtained with potassium chloride and sodium lauryl sulfate or sodium heptadecyl sulfate. However, detergents which were not synergistic with neomycin, polysorbate 80, and oxyethylene-oxypropylene polymer, did not affect the osmotic pressure resistance. Increased cell wall permeability in the presence of endomycin is believed to be a factor in endomycin-neomycin synergism.

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