ABSORPTION OF NYSTATIN BY MICROORGANISMS

J. O. LAMPEN, ELLIOTT R. MORGAN, ADELAIDE SLOCUM, AND PETER ARNOW

The Squibb Institute for Medical Research and the Institute of Microbiology, Rutgers, the State University, New Brunswick, New Jersey

Received for publication February 18, 1959

Nystatin, a polyene antibiotic produced by *Streptomyces noursei* (Brown and Hazen, 1957), is highly active in inhibiting the growth of a wide variety of fungi, but has little or no effect on other types of microorganisms or on animal cells. It inhibits both endogenous metabolic activity and the utilization of various substrates by fungi, especially yeast (Lampen *et al.*, 1956, 1957; Harmon and Masterson, 1957; Meyer-Rohm *et al.*, 1957; Peynaud *et al.*, 1957; Bradley, 1957, 1958). This derangement of metabolism appears to bear a direct relation to the fungicidal process, since it was observed (Lampen *et al.*, 1957) that carbohydrate utilization by microorganisms was blocked by nystatin only if their growth was sensitive to the antifungal agent. The inhibition of metabolic activity by nystatin could be prevented if a boiled extract of yeast was present, but reversal of an existing inhibition was not achieved.

It was previously reported that materials present in baker's yeast removed nystatin from aqueous solution (Lampen *et al.*, 1957). The subject of the present investigation is the nature of this absorption of nystatin by microorganisms and its importance as a factor in the agent's unique specificity for fungi.

MATERIALS AND METHODS

The organisms used in this study, the growth conditions employed, and the experimental procedures were, in general, those described by Lampen *et al.* (1957). *Streptomyces griseus* (I.M. strain 3478) and *Streptomyces lavendulae* (ATCC strain 8665) were grown for 6 to 7 days at 25 C on the glucose-amino acid-salts medium of O'Brien *et al.* (1952). For *Pythium debaryanum* (ATCC strain 10393) the growth medium contained, per L: tryptone, 0.5 g; malt extract, 0.3 g; yeast extract, 0.3 g; and glucose, 1.0 g. After growth in stationary culture for 7 to 10 days at 25 C, the resulting mat was washed and cut into pieces ½ cm square for testing. The commercial baker's yeast was freed of starch before use by centrifuging 4 times from 10 volumes of distilled water and then was resuspended in the appropriate buffer. All cell weights represent dry matter after drying overnight at 105 C. Unless otherwise stated, the nystatin samples were commercial crystalline preparations with a potency of 2800 to 3000 units per mg (Pagano and Stander, 1955). The highly purified preparation of nystatin (HV-917; potency, 5550 units per mg) was generously furnished by Dr. J. D. Dutcher of the Squibb Institute for Medical Research. The use of the readily available commercial preparation for most of our experiments, rather than the highly purified material, seems justified since, starting with equal absorbencies, both compounds gave identical results.

The following procedure was generally used to measure the uptake of nystatin by cells. A solution of nystatin in 0.2 ml of 1 per cent aqueous dimethylsulfoxide was added to 2.0 ml of aqueous cell suspension (4.5 to 5.5 mg of cells), 2.0 ml of pH 4.5 succinate-phosphate buffer (other buffers 0.05 M), and 1.8 ml of water. The addition of nystatin was considered zero time. The mixture was incubated at 30 C for various periods of time, chilled rapidly, and the cells removed by centrifugation at 2500 × G for 15 min. Nystatin was estimated in the supernatant fluid by measurement of its absorption at 321 mμ. The absorbency values of the control solutions without cells did not change over a 2-hr period. In test mixtures with cells, the material in the supernatant solution absorbing at 321 mμ could still be extracted completely with butanol at the end of the 2-hr incubation period. Thus there does not appear to be significant leakage of cell materials which absorb at 321 mμ. The nystatin disappearing from the supernatant fluid was assumed, therefore, to have been absorbed and is recorded as micrograms removed per milligram of cells. Evidence is presented that this disappearance represents primarily a true uptake and not destruction of the nystatin.

In figure 1 are presented the absorption spectra

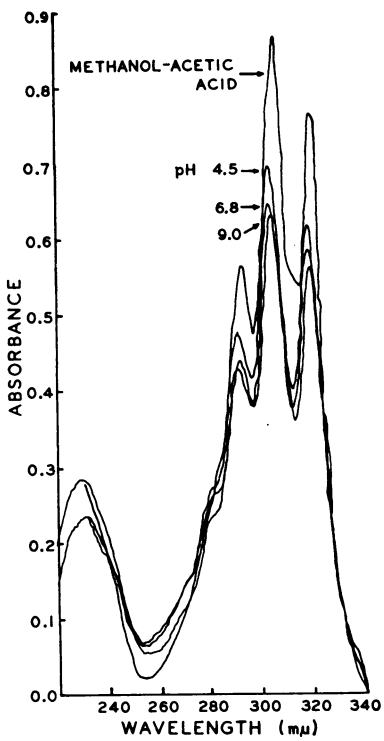

Figure 1. The absorption spectra of purified nystatin (sample HV-917; 10 μg per ml final concentration) in methanol-0.05 per cent acetic acid, pH 4.5 succinate-phosphate buffer, pH 6.8 phosphate buffer, and pH 9.0 carbonate-bicarbonate buffer. For the three aqueous media the conditions are essentially those used for the measurement of absorption (Materials and Methods). A solution of nystatin in 0.2 ml of 1 per cent aqueous dimethylsulfoxide was diluted with 2 ml of the appropriate buffer and water to 6 ml and the spectrum determined with a Cary model 14 recording spectrophotometer.

of purified nystatin (sample HV-917) in (a) methanol-0.05 per cent acetic acid (Dutcher *et al.*, 1953) and (b) under the test conditions generally used for measuring the uptake of nystatin by cells. The spectra in aqueous media are similar to the spectrum in methanol-acetic acid, but the extinction values are lower. There is a shift in the maxima from 292, 305, and 319 mμ in methanolic solution to 293, 306, and 321 mμ in aqueous media.

From the low extinction values in aqueous media, it appears probable that nystatin is present as micelles and is not in true solution. This is also indicated by the fact that nystatin is not dialyzable under these conditions even though its formal molecular weight is only approximately 950. Thus in several trials at pH 4.5 and pH 6.8 with concentrations of 10 to 30 μg of nystatin per ml of 0.1 per cent aqueous dimethylsulfoxide solution, less than 5 per cent of the nystatin was dialyzed during 24 hr at 4 C or 37 C. The rate of dialysis was not increased in the presence of 3 per cent NaCl. In these tests, 20 ml of solution in 1-in tubing was dialyzed with stirring against 10 volumes of the same solution. The nystatin could be recovered unaltered from the dialysis bag at the end of the experiment.

Since nystatin does not appear to be in true solution, theoretical interpretation of the data becomes complex. It may also be predicted that the presence of lipid-soluble or surface-active substances could alter sharply the micellar structure and hence the effective concentration of nystatin. Nevertheless under constant conditions, the results are reproducible and interpretation of the data in an empirical manner seems warranted.

RESULTS

Absorption by various microorganisms. Microorganisms whose growth is sensitive to nystatin (*Saccharomyces cerevisiae*, *Candida albicans*, and *Penicillium chrysogenum*) absorbed significant amounts of the antibiotic (table 1), whereas the insensitive organisms (*Streptococcus faecalis*, *Escherichia coli*, the two actinomycetes, and the phycomycete *P. debaryanum*) did not remove detectable quantities. Only one resistant strain, *C. albicans* strain R was tested. The concentration of nystatin required to inhibit growth or glycolysis of this strain was severalfold that required with the parent *C. albicans* strain S. At a given concentration of nystatin, the amount absorbed by the R strain was definitely less than with the S strain. Thus within the limited series studied, there is a correlation between the ability of an organism to remove nystatin from solution and its sensitivity to the antibiotic.

Characteristics of the absorption. For a detailed study of the factors affecting absorption, commercial baker's yeast (strain A-B) was used. With this material both the rate and the total uptake of nystatin increased as the concentration was raised from 2.5 to 15 μg per ml (figure 2*A*). In some experiments the relation between the maximal amount absorbed and the concentration

TABLE 1

Absorption of nystatin by microorganisms

Experimental conditions described under Materials and Methods, 30 min incubation

Organisms	Nystatin Conc	Nystatin Absorbed	Minimal Growth Inhibitory Conc*
	μg/ml	μg/mg cell	μg
Saccharomyces cerevisiae strain F	1.7	0.44	3
	8.3	1.60	
S. cerevisiae strain LK2G12	1.7	0.50	3
	8.3	1.90	
S. cerevisiae strain A-B	10.0	3.00†	—
Candida albicans strain S	4.0	0.50	4
	8.0	2.20	
	24.0	6.80	
C. albicans strain R	4.0	0.17	12
	8.0	1.70	
	24.0	5.90	
Penicillium chrysogenum strain Q176	1.7	0.10	5
	8.3	1.60	
Streptococcus faecalis strain 10C1	10.0	0.20	>100
Escherichia coli strain ATCC 9723	3.3	<0.50	>100
	6.7	<0.50	
Streptomyces griseus	8.3	<0.50	>100
Streptomyces lavendulae	8.3	<0.50	>100
Pythium debaryanum	8.3	<0.50	1000‡

* *Unpublished data*, courtesy of H. A. Stout. Procedure that of Stout and Pagano (1956).

† 40 min incubation.

‡ *Unpublished data*, courtesy of F. Pansy.

was essentially linear. In others the maximum appeared to be a function of the log of the concentration. The question should probably be reexamined with the aid of radioactive nystatin, since with the present method the concentration range which can be used is narrow.

It was previously reported (Lampen *et al.*, 1957; Peynaud *et al.*, 1957) that nystatin is more effective in inhibiting glycolysis at an acid pH (4.5) than at neutrality. From the results shown in figure 3 one may suggest that the apparent effect of pH upon the inhibition of glycolysis is actually a reflection of differences in the absorption of nystatin at pH 4.5 and 6.8. As can be seen from the curve for 20 min, the quantity absorbed rapidly at pH 4.0 was severalfold that at pH 6 and above. This quantity is not a function of the specific buffers employed. Comparable values were obtained at pH 4.5 with phthalate and with succinate-phosphate buffers. At pH 6.8 the phosphate buffer could be replaced by pyrophosphate or maleate buffers without affecting the results. Following this initial absorption of nystatin, a slower uptake continues. With 10 μg of nystatin per ml, the absorption at pH 6.0 and above did not exceed 2 μg per mg but this does not represent a saturation of available sites, since with 20 μg per ml, a value of 3.5 μg per mg was observed.

The group concerned in the initial rapid uptake has an apparent pK_a of 4.5 to 5.0, which approximates that of the carboxyl group of nystatin (J. D. Dutcher, *unpublished observations*). Since yeast cells are known to be permeable only to the nonionized forms of fatty acids (Conway and Downey, 1950), it is probable that the absorption of nystatin also involves primarily the nonionized molecule.

The effect of temperature was complex (figure 2*B*). At 4 C there was no detectable uptake. Absorption occurred at 30 C and the rate increased as the temperature was raised to 45 C and to 55 C. At 55 C, absorption ceased abruptly after 10 min. This suggested the possibility that a component required for the transport of the drug across the cell membrane was inactivated at 55 C. However, when cells were incubated at 55 C for 30 min prior to the addition of the nystatin, the curve of uptake of the antibiotic during the subsequent period at 55 C approximated that shown in figure 2*B* for fresh cells. It was also observed that cells incubated at 55 C with nystatin until absorption had ceased were able to take up more nystatin if the temperature was adjusted to 40 C (figure 2*B*) or if the concentration of nystatin was doubled.

Since the system for the uptake of nystatin appeared relatively heat-resistant, a suspension of yeast cells was held in a boiling water bath for 5 min and then tested. The boiled cells absorbed the antibiotic more rapidly than did the intact cells (table 2). It should also be noted that the

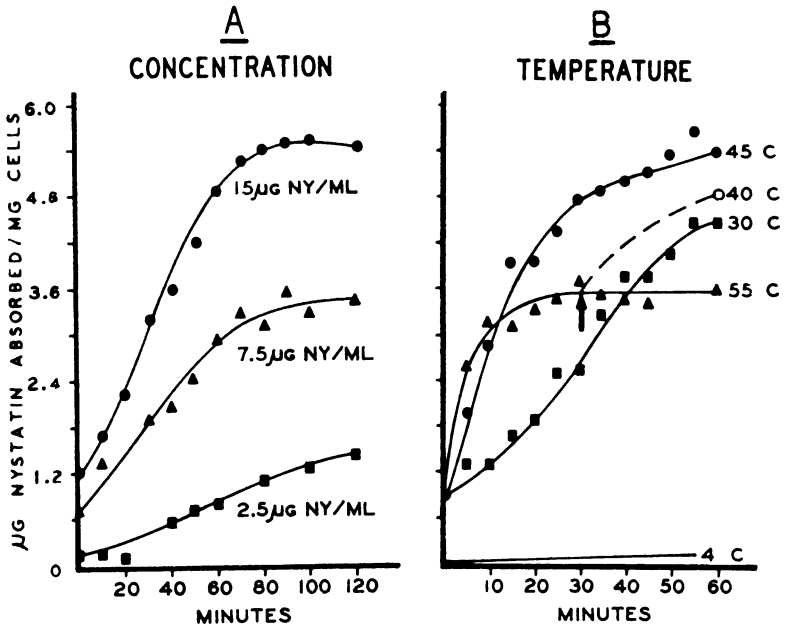

Figure 2. Effect of concentration and temperature on the uptake of nystatin by yeast (strain A-B) Unless otherwise indicated, the conditions were: final concentration of nystatin 10 μg per ml, temper ature 30 C, and pH 4.5 succinate-phosphate buffer. Other experimental conditions were as described under Materials and Methods. After 30 min incubation, a portion of the test mixture at 55 C was placed in a 40 C water bath (arrow, graph *B*). The absorption by this sample during the following 30 min is indicated by the broken line.

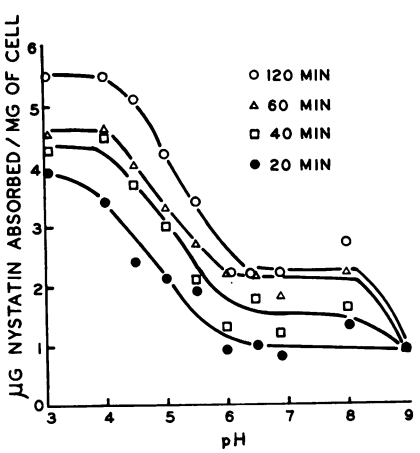

Figure 3. The absorption of nystatin by yeast (strain A-B) as a function of pH. The concentration of nystatin was 10 μg per ml. At pH 4.5 the succinate-phosphate buffer was used, at lower pH the phthalate buffers of Clark and Lubs (1917), and at pH 5.0 and above their phosphate buffers. The pH of the test mixtures did not change detectably during incubation. Other details as given in Materials and Methods.

TABLE 2

*Characteristics of the uptake of nystatin by boiled yeast cells**

	pH	Temp	Nystatin Absorbed		
			1 Min	20 Min	60 Min
		C	μg/mg cells		
Intact cells	4	4	0.0	0.0	0.2
		30	0.4	2.4	3.5
		45	0.6	2.7	2.9
	7	30	0.2	0.6	1.2
Boiled cells†	4	4	2.6	5.1	5.8
		30	4.5	5.7	5.9
		45	1.8	2.7	3.1
	7	30	2.1	3.6	4.2

* Nystatin (sample HV-917) concentration was 10 μg per ml; strain A-B yeast was used. Other experimental procedures as given in Materials and Methods.

† An aqueous suspension of washed yeast (5.0 mg dry weight per 2 ml) was incubated in a boiling water bath for 5 min, cooled, and treated as above.

TABLE 3

Requirement of energy for the absorption of nystatin by yeast

The experimental conditions were those described in Materials and Methods for the measurement of absorption. The Warburg respirometer was used and the reagent volumes were one-half those generally employed. The gas phase was 95 per cent N_2-5 per cent CO_2. Glucose and nystatin were added at zero time. After 60 min the cup contents were used for the estimation of nystatin uptake.

Nystatin	Glucose	CO_2 Production	Nystatin Absorbed
μg/ml	*M*	*μL/60 min*	*μg/mg cells*
0	0.02	870	0.0
3.3	0.02	310	1.9
0	0	14	0.0
3.3	0	38	0.1

character of the uptake has been altered in that it is now much less dependent on temperature or pH.

The maximal amount absorbed by the boiled cells at 45 C was significantly less than the value obtained at 0 or 30 C. A similar result was obtained with intact cells at 55 C (figure 2) and 45 C (table 2). This may indicate that micellar size, and therefore the total amount of nystatin which can attach to a cell, is less at 45 to 55 C than at 30 C.

Absorption of nystatin did not occur at 30 C in the absence of a metabolizable substrate (table 3). The yeast preparation does not have significant endogenous anaerobic metabolism and under these conditions nystatin was not absorbed. Uptake occurred anaerobically when glucose was added. In the presence of air (as in the standard procedure) the oxidation of endogenous reserved was adequate to support binding.

Absorbed nystatin was not removed by repeated washing of the cells with buffer or acetone, or by adjusting the pH of the cell suspension from 4.5 to 6.8. It could be extracted by shaking the cell paste at steambath temperature with 50 per cent (v/v) aqueous isopropanol. If the cells from a standard test mixture were extracted twice for 5 min with 3.0 ml volumes of aqueous isopropanol, the "nystatin" recovered (based on E_{321} values) was 90 to 100 per cent of that which had disappeared from the original solution. The spectrum of the extracted material was essentially identical with that of the original antibiotic. However, the nystatin concentrations of these extracts as determined by bio-assay (Pagano and Stander, 1955) were only about 60 per cent of those calculated from their extinctions at 321 mμ. There did not appear to be any alteration in the specificity of the extracted material since a concentration of 2.5 μg per ml (calculated from E_{321}) gave essentially the same lag in inhibiting yeast metabolism as did this concentration of authentic nystatin (Lampen *et al.*, 1957). Also, glycolysis by the insensitive organism *S. faecalis* (strain 10C1) was not prevented in the presence of 12 μg of the extracted material per ml. Therefore, it is concluded that the bulk of the absorbed nystatin was recovered unchanged. The nature of the apparent losses in biological activity cannot be defined at present, but they may result from the known chemical instability of the agent.

Characteristics of fungicidal action. Since the rate and extent of absorption were altered by variations in temperature and pH, these parameters were utilized to explore the relationship be-

TABLE 4

*Effect of temperature and pH on the killing of yeast by nystatin**

pH	Temp	Survival† after Exposure to Nystatin for:	
		10 Min	30 Min
	C	%	%
4.5	4	100	70
	30	33	0.55
	45	0.1	0.1
6.8	30	42	5.5
	45	0.1	0.1

* Each test mixture contained 5.0 mg yeast, 2.0 ml of pH 4.5 succinate-phosphate buffer or of pH 6.8 sodium-potassium phosphate buffer (0.05 M), 50 μg of nystatin in 0.2 ml of 1 per cent aqueous dimethylsulfoxide, and water to 6.0 ml. At the end of the incubation period, a 1.0-ml sample was diluted 100-fold with ice-cold sterile water to prevent further absorption of nystatin. Viable counts were made by standard procedures using Wort agar (Difco) (growth period 24 hr at 37 C). To suppress the growth of bacteria, 20 μg of tetracycline was incorporated into each plate.

† Relative to control samples without nystatin. Viable counts performed by Mr. F. Pansy.

tween absorption and the lethal action of the antibiotic. As is indicated in table 4, yeast cells were not killed at 4 C by a concentration of nystatin which was rapidly lethal at 30 or 45 C. Thus killing did not occur in the absence of absorption. The fungicidal action at 30 C was definitely slower at pH 6.8 than at pH 4.5, but no effect of pH was detected at 45 C. It was observed that the absorption could be halted at any time by diluting the test mixtures 100-fold with water at 2 C and holding the samples at this temperature until they were mixed with wort agar for plating.

DISCUSSION

The absorption of nystatin by intact yeast appears to require metabolic energy since uptake did not occur at 0 C or in the absence of an energy source. Boiled cells did not show this requirement. One may, therefore, suggest that the absorption has it least two distinct phases: (a) A rapid and tenacious binding by a substance(s) in the cell which is relatively stable to heat. This binding does not show major dependence on pH over the range studied. (b) Access to this substance is determined by a system which requires energy, and whose activity is dependent on pH and on the concentration of nystatin in the external medium. It should be noted, however, that one cannot exclude the alternative possibility that boiling has produced an actual change in the characteristics of the absorbing system. This question is under investigation.

The characteristics of the absorbing system are consistent with the bulk of the present data concerning both the fungicidal effect of nystatin and its action on metabolism. Sugar utilization is more sensitive to nystatin at acid than at neutral pH (Lampen *et al.*, 1957). This may be the result of the enhanced absorption of the antibiotic under acid conditions. The effect of variation in pH on the killing of yeast was much less dramatic, but in several experiments the death rate at pH 4.5 was definitely greater than at pH 6.8. Peynaud *et al.* (1957) reported that nystatin inhibited growth more effectively in acid than in neutral media. We have suggested (Lampen *et al.*, 1957) that the rate of uptake may be more critical in relatively short metabolic studies than in 16- or 24-hr growth tests. The total amount which can be absorbed may also be less important in the growth studies, since cells can absorb considerably more nystatin than is required to prevent the formation of colonies.

The amount of nystatin which can be taken up by sensitive cells is surprisingly large. The maximum we have observed is equivalent to 6 μg of the highly purified preparation per mg of cells, or 0.6 per cent of the cell weight. A 6-μg amount of nystatin represents 3.8×10^{15} molecules (molecular weight = 948 (Dutcher *et al.*, 1953)), and our commercial yeast has approximately 5.7×10^{8} cells per mg dry weight. Thus each yeast cell can absorb at least 7×10^{6} molecules of nystatin.

The results presented in table 4 show clearly that there is no fungicidal action at 4 C, at which temperature uptake of nystatin does not occur. Similarly, both absorption and the lethal effects are accelerated when the temperature is raised from 30 to 45 C. Of the single sensitive:resistant pair studied, *C. albicans* strain R absorbs less nystatin than does the S strain under comparable conditions. Preliminary results with other resistant Candida strains also indicate a lowered uptake by resistant organisms (J. O. Lampen and P. Arnow, *unpublished observations*).

Most of the results presented in this paper were obtained before we examined in detail the effects of pH on the absorption of nystatin. The buffer system (succinate-phosphate, pH 4.5) used in most of this work is unfortunately close to the pK_a of the group concerned in the initial absorption. For subsequent experiments, a pH 4.0 phthalate or succinate-phosphate buffer should be preferable.

The data obtained in this study are consistent with the hypothesis that absorption by the fungal cell is an essential step in the action of nystatin and is a critical factor in determining sensitivity to the agent. This hypothesis can be tested by determining what effect agents which alter the uptake of nystatin have on its fungicidal and metabolic actions. This work is in progress.

A number of other antibiotics are known to be bound by microbial cells. Pledger (1957) reported that candidin, another polyene antifungal agent, was bound by strains of *C. albicans* and *S. cerevisiae*, but not by strains of *Staphylococcus aureus*, *E. coli*, or *Bacillus subtilis*. The latter organisms are not sensitive to candidin. Pledger suggested that this binding may be important in determining the specificity of candidin. The binding of penicillin by bacterial cells has been

studied by several groups (Maass and Johnson, 1949*a*, *b*; Rowley *et al.*, 1950; Daniel and Johnson, 1954). Cooper (1956) has reviewed the evidence that the binding reaction is an essential element in the action of penicillin. It should be noted, however, that variation in binding capacity is only one of many factors which can alter sensitivity to penicillin (see Cooper, 1956, for a discussion of this question).

Videau (1958) has demonstrated the fixation of a variety of antibiotics by staphylococci. He suggests that this may be of special importance in the action of spiramycin, carbomycin, streptogramin, and erythromycin.

SUMMARY

Nystatin was removed from aqueous solution by yeasts and other fungi whose growth was inhibited by the antibiotic. This was not observed with a group of insensitive organisms.

The absorption of nystatin by intact cells was dependent on the concentration of nystatin, on temperature, and on pH. No uptake occurred at 0 to 4 C. At acid pH, nystatin was absorbed more rapidly and in a greater final amount than at pH 6 and above. Absorption by boiled cells was extremely rapid and showed less dependence on temperature and pH than is characteristic of intact cells. Absorbed nystatin could not be removed by washing the cells with buffer or with acetone or by changing the pH of the suspending medium. The major portion could be recovered, apparently unaltered, by extraction with 1:1 aqueous isopropanol.

In the absence of a substrate which could be metabolized, absorption of nystatin by yeast cells did not occur at 30 C. Uptake occurred when glucose was present or if oxidation of endogenous reserves was permitted. Therefore, it is postulated that the uptake involves both an initial penetration of or attachment to the cell, this phase requiring energy and dependent on pH, and a subsequent binding by cell constituents which can only be reversed with difficulty.

Uptake and fungicidal action were similarly affected by changes in temperature, pH, or concentration of nystatin, although the amount of the antifungal agent which could be absorbed was considerably in excess of that required to kill the cells.

The results are consistent with the hypothesis that absorption by the fungal cell is an essential step in the action of nystatin and is a critical factor in determining sensitivity to the agent.

REFERENCES

BRADLEY, S. G. 1957 Effect of nystatin on *Candida stellatoidea*. Antibiotics & Chemotherapy, **8**, 282–286.

BRADLEY, S. G. 1958 Interactions between phosphate and nystatin in *Candida stellatoidea*. Proc. Soc. Exptl. Biol. Med., **98**, 786–789.

BROWN, R. AND HAZEN, E. L. 1957 Present knowledge of nystatin, an antifungal antibiotic. Trans. N. Y. Acad. Sci., **19**, 447–456.

CLARK, W. M. AND LUBS, H. A. 1917 The colorimetric determination of hydrogen ion concentration and its applications in bacteriology. J. Bacteriol., **2**, 1–34.

CONWAY, E. J. AND DOWNEY, M. 1950 An outer metabolic region of the yeast cell. Biochem. J., **47**, 347–355.

COOPER, P. D. 1956 Site of action of radiopenicillin. Bacteriol. Revs., **20**, 28–48.

DANIEL, J. W., JR. AND JOHNSON, M. J. 1954 Properties of the penicillin binding component of *Micrococcus pyogenes*. J. Bacteriol., **67**, 321–328.

DUTCHER, J. D., BOYACK, G., AND FOX, S. 1953 The preparation and properties of crystalline fungicidin (nystatin). In *Antibiotics annual 1953–1954*, pp. 191–194. Medical Encyclopedia, Inc., New York.

HARMAN, J. W. AND MASTERSON, J. G. 1957 The mechanism of nystatin action. Irish J. Med. Sci., No. **378**, 249–253.

LAMPEN, J. O., MORGAN, E. R., AND SLOCUM, A. C. 1956 Inhibition of sugar utilization by nystatin. Federation Proc., **15**, 295.

LAMPEN, J. O., MORGAN, E. R., AND SLOCUM, A. C. 1957 Effect of nystatin on the utilization of substrates by yeast and other fungi. J. Bacteriol., **74**, 297–302.

MAASS, E. A. AND JOHNSON, M. J. 1949*a* Penicillin uptake by bacterial cells. J. Bacteriol., **57**, 415–422.

MAASS, E. A. AND JOHNSON, M. J. 1949*b* The relations between bound penicillin and growth in *Staphylococcus aureus*. J. Bacteriol., **58**, 361–366.

MEYER-ROHM, J., HOPFF, W., AND LANGE-BROCK, T. 1957 Experimentelle Untersuchungen über Wirkungsweise und therapeutische Effekte von Nystatin. Arzneimittel-Forsch., **7**, 355–357.

O'BRIEN, E., WAGMAN, G. H., AND PERLMAN, D. 1952 Synthetic media for growth and streptomycin production by *Streptomyces griseus*. Bacteriol. Proc., **1952**, 25.

PAGANO, J. F. AND STANDER, H. 1955 Bioassay of nystatin (Mycostatin) in body fluids. In *Therapy of fungus diseases: an international*

symposium, pp. 186–194. Edited by T. H. Sternberg and V. D. Newcomer. Little, Brown and Co., Boston.

PEYNAUD, E., LAFOURCADE, S., AND LAFON, M. 1957 Action de nouveau antibiotiques antifongiques sur les levures de vins. Compt. rend., **244**, 2426–2429.

PLEDGER, R. A. 1957 Biological and biochemical properties of polyene antibiotics, pp. 66–77. Ph.D. thesis, Rutgers University, New Brunswick, New Jersey.

ROWLEY, D., COOPER, P. D., ROBERTS, P. W., AND SMITH, E. L. 1950 The site of action of penicillin. 1. Uptake of penicillin on bacteria. Biochem. J., **46**, 157–161.

STOUT, H. A. AND PAGANO, J. F. 1956 Resistance studies with nystatin. In *Antibiotics annual 1955–1956*, pp. 704–710. Medical Encyclopedia, Inc., New York.

VIDEAU, D. 1958 Sur le mode d'action des antibiotiques cas particulier de la Spiramycine. Ann. inst. Pasteur, **94**, 709–723.