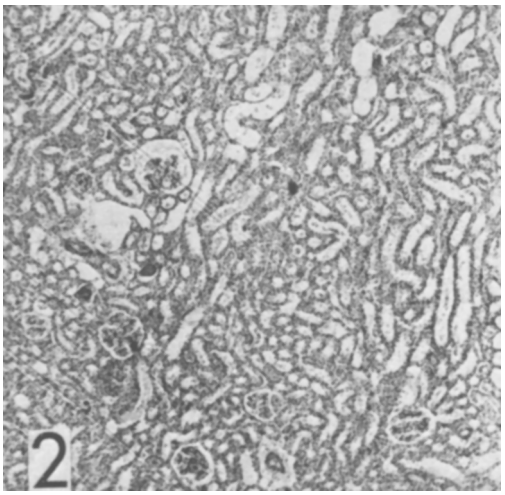

FIG. 2. Kidney from intact rat. H & E × 55.

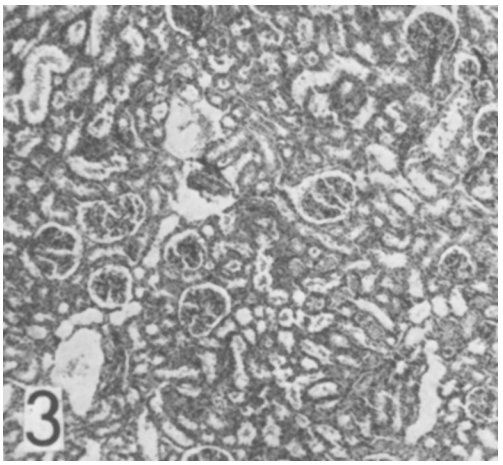

FIG. 3. Kidney from adrenalectomized rat. H & E × 55.

intact animal. Evidences of renal damage in intact animals, however, were not present in these which had previously undergone hypophysectomy. Pathological changes which occurred in kidneys of the adrenalectomized group were similar to those described in normal rats, but less severe. Whether for direct or indirect reasons absence of functioning hypophysis was associated with tolerance of renal tissue toward aminonucleoside even though the nephrotic syndrome was clinically similar.

Summary. Nephrosis was induced in immature rats by subcutaneous administration of aminonucleoside. Subjects were divided in 3 groups: I, hypophysectomized, II, intact and III, adrenalectomized. All 3 groups developed proteinuria and ascites. Examination of stained sections, however, showed that kidneys of intact rats had undergone marked deterioration. Pathological changes were less marked in the adrenalectomized group, and absent in the hypophysectomized group.

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Significance of Nystatin Uptake for Its Antifungal Action. (25098)

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Nystatin, a polyene antifungal agent produced by *Streptomyces noursei*(1), inhibits growth and utilization of various substrates by fungi, especially yeasts(2). It was previously observed(3) that nystatin is removed from solution by organisms whose growth is sensitive to its action, but not by insensitive organisms. The properties of this uptake appeared to correlate with available information both on specificity of nystatin and on its fungicidal and metabolism-inhibiting actions. It was suggested, therefore, that this uptake is essential for nystatin action and is a critical factor in determining sensitivity. To evalu-

ate more precisely the importance of absorption of nystatin, we examined a series of sensitive : resistant pairs of *Candida* strains(4,5). We have also studied the effect of agents which alter uptake of nystatin by yeast and the consequent alteration of its fungicidal and metabolic action. Sterols(6), bile salts(7), and high concentrations of phosphate(5) have been reported to reduce biological effectiveness of nystatin or related polyene antifungals. The relation of certain of these agents to the absorption process has also been examined. A separate publication concerning effect of sterols on action of several polyene antifungal antibiotics is in preparation.

Methods. Absorption tests were performed as previously described(3). Unless otherwise stated, nystatin was dissolved in 0.2 ml of 1% aqueous dimethyl sulfoxide. To this volume were added the appropriate buffer, 5 mg dry weight of *Saccharomyces cerevisiae* or *Candida* strains and water to 6 ml. The buffers were 0.067 M pH 4.0 phthalate or pH 6.8 to 7.0 phosphate(8), or a 0.017 M succinate-0.017 M phosphate buffer at pH 4.5. The tubes were incubated with occasional shaking at 30°C, and after periods indicated were chilled and centrifuged at 2500 x g for 15 minutes. Absorbance at 321 mμ was used as a measure of nystatin content of supernatant fluids. In certain experiments the absorption tests were performed in Warburg flasks to permit a better comparison with metabolic measurements. Absorption is more rapid under these conditions, perhaps because there is better contact between nystatin and cells. However, the basic characteristics of the absorption are unchanged. Manometric studies were carried out at 30°C, using standard Warburg technics. For measurement of oxygen uptake, the gas phase was air. Unless otherwise stated, each cup contained phthalate buffer, pH 4, 2.5 mg dry weight of cells (washed 4 times in water), the antifungal agent in 0.1 ml of 1% aqueous dimethyl sulfoxide, 60 μM of glucose, and water to 2.8 ml. The center well contained 0.2 ml of a 10% KOH solution. For anaerobic studies the gas phase was 95% N_2 and 5% CO_2. Unless otherwise stated, nystatin, glucose, zymosan, serum albumin, or inhibitors were added at 0 minutes. The nystatin was either a highly purified crystalline preparation with biological activity(9) of approximately 5500 units/mg (Squibb Lots HV-917 or HV-942), or the commercial crystalline product with biological activity of 2850 units/mg (Lot 15289-011). These products and the methyl ester and N-acetyl derivative of nystatin were kindly furnished by Dr. J. D. Dutcher, Squibb Institute for Medical Research. The methyl ester and N-acetyl derivative have no antifungal activity either *in vivo* (oral dosage) or *in vitro* (Dutcher, J. D., *et al.*, unpublished observations). Zymosan was washed 4 times and resuspended in water before use. An analysis of this preparation (No. 6B14) is given by Di Carlo and Fiore(10). The sensitive : resistant pairs of organisms described by Littman *et al.*(4) were generously provided by Dr. J. Pagano, Squibb Institute. Dr. S. G. Bradley, University of Minnesota, furnished the *Candida stellatoidea* strains(5). These cells were grown overnight at 28°C with shaking in Penassay broth (Difco) plus 1% glucose. The cells were washed 4 times in distilled water and resuspended in water. The *S. cerevisiae* was commercial bakers' yeast from Anheuser-Busch. The term yeast, when not further defined, refers to this material.

Results. The time sequence for action of nystatin on metabolism is significant, since it affords both an indication of the manner in which nystatin acts and of the changes expected when uptake of nystatin is prevented or reduced. Fig. 1 presents a typical test, using inhibition of glycolysis as the measure of nystatin action. Analogous results are obtained if respiration is the criterion. It should be noted that the rate of glycolysis is normal or even elevated until a sudden drop in activity occurs. Within a short period, gas production generally ceases completely. It has not been possible to reverse this loss of activity once it has occurred. Although higher concentrations of nystatin are more likely to cause an initial stimulation than are lower levels, the primary effect of concentration is to determine the time required before the sharp drop in activity takes place. There is also a rough inverse proportionality between time required for inhibition and quantity of

TABLE I. Effect of Nystatin on Sensitive : Resistant Pairs of Candida Strains.*

Organisms	Ratio of fungicidal conc., R/S†	Conc. of nystatin inhibiting glucose oxidation			Absorption of nystatin (10 μg/ml) in 60 min.		
		S	R	R/S	S	R	R/S
		μg/ml					
Candida guilliermondii	>4 ‡	3	16	5	5.1	1.3	1:4
C. parakrusei	1.6‡	9	13	1.4	2.8	2.1	1:1.3
C. tropicalis	13.3‡	2	8	4	5.4	3.5	1:1.5
C. stellatoidea	12 §	1.3	8	6	5.4	3.6	1:1.5

* The commercial nystatin preparation and pH 4.5 succinate-phosphate buffer were used in all tests.
† Ratio of values for resistant strain (R) and for parent sensitive culture (S).
‡ Data of Littman *et al.*(4).
§ Data of Bradley(5).

nystatin absorbed by the cells.

If nystatin uptake is essential to its action, an agent which reduces or delays this uptake should extend the period during which metabolism appears normal, and thus will appear to furnish complete protection during this time. If inhibition of uptake is not complete, a rapid loss of metabolic activity will eventually occur and cannot then be reversed. These general principles will be used in assessing the experiments which follow.

Table I summarizes comparative tests with pairs of nystatin-resistant : sensitive strains of *Candida*. From the resistant : sensitive ratios presented, it can be seen that the reported resistance of these strains(4,5) to fungicidal and fungistatic action of nystatin was paralleled by increased resistance of their oxidative metabolism. The concentrations of nystatin which inhibit oxidation approximated those required to prevent growth, as observed previously(3). The resistant strains all absorbed less nystatin than did comparable sensitive strains, but there was no quantitative correlation between magnitude of changes in uptake and in sensitivity. For example, differences in uptake with *C. tropicalis* and *C. stellatoidea* appear too small to account for the changes in sensitivity.

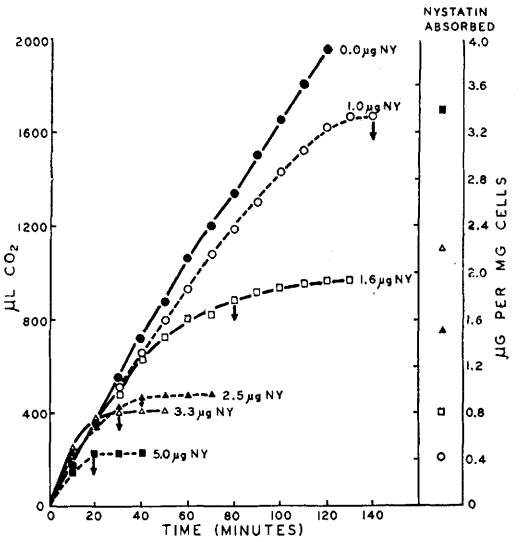

FIG. 1. Action pattern of nystatin (NY) on glycolysis. Final volume = 3.0 ml. Purified nystatin used; values on graph represent μg/ml. At the arrow (↓) metabolism is considered to have ceased. Amount of nystatin absorbed in 30 min., as determined in parallel series of vessels, is represented in column at right (symbols as in graph of CO_2 production).

TABLE II. Glycolysis and Absorption of Nystatin by *S. cerevisiae* in the Presence of Serum Albumin.

Nystatin,* μg/ml	Serum albumin, mg/ml	CO_2 production, μl/60 min.	Metabolic activity ceased, min.	Nystatin absorbed, μg/mg yeast 60 min.†
0	0	990	>120	
5	0	65	15	4.6
0	32	1040	>120	
5	12	435	35	2.0
5	19	540	45	1.0
5	32	810	75	.4
21	32	81	20	

* Purified preparation.
† Absorption essentially complete at 20 min.

High levels of human serum albumin decreased absorption of nystatin by yeast and protected glycolysis against its inhibitory action (Table II). This protection approximates that expected on the basis of reduction in nystatin uptake (Fig. 1).

Zymosan, a yeast fraction shown by Di Carlo and Fiore(10) to consist primarily of yeast cell ghosts, is a potent binding agent for

TABLE III. Effect of Zymosan on Glycolysis by *S. cerevisiae*.

Nystatin* added, μg/ml	Zymosan, mg/ml	μl CO_2/60 min.	Metabolism ceased, min.	Residual nystatin at 10 min., μg/ml†
0	0	910	>120	0
3.5	0	210	25	3.5
"	1.1	264	25	2.7
"	3.5	815	65	1.4
"	10.7	965	>120	.4

* Purified nystatin used. Buffer was pH 4.5 succinate-phosphate.

† Parallel test series without cells to indicate magnitude and rapidity of removal of nystatin by zymosan.

nystatin. Yeast glycolysis can be protected by zymosan and this protection can be explained readily by rapid removal of nystatin by the zymosan (Table III). Neither zymosan nor serum albumin will reverse the action of nystatin once inhibition of metabolism has occurred.

It was of interest to know if biologically inactive derivatives of nystatin would be absorbed by yeast. It was found that amounts of methyl ester and N-acetyl derivative absorbed by intact yeast and zymosan at pH 4.0 or 6.8 were much less than the uptake obtained with nystatin itself. Values for absorption were generally within experimental error of procedure. A definite uptake—approximately 25% of nystatin absorbed under the same conditions—was demonstrated only with methyl ester-intact yeast combination at pH 4.0.

Bradley(5) reported that 0.5 M phosphate prevented partial inhibition of oxidation in *C. stellatoidea* which he obtained with 20 μg of nystatin/ml. This concentration of phosphate did inhibit uptake of nystatin by yeast by approximately 60% under our conditions; however, it is toxic to glucose oxidation by *S. cerevisiae* and actually increased its sensitivity to nystatin (unpublished data). Thus no direct comparison between our results and those of Bradley can be made.

Schneierson *et al.*(7) observed that addition of 1% bile salts to the medium reduced sharply the activity of nystatin in inhibiting growth of *C. albicans*. The commercial preparation of bile salts used by these authors interfered in our optical test for binding. Purified sodium desoxycholate could be tested at pH 7 (precipitation at pH values below 6), and under these conditions reduced nystatin uptake by 80 to 90%. Since bile salts are known to form inclusion complexes(11), one may suggest that they reduce the effective concentration of nystatin and thus reduce its activity without direct metabolic interaction.

Uranyl ions are known to combine with the surface of intact yeast but not to penetrate the cell. They inhibit uptake of sugars by yeast(12). At 1 x 10^{-4} M, uranyl acetate inhibited uptake of nystatin by 60 to 80%. This concentration reduced the rate of oxidation of glucose by yeast but effected almost complete protection of residual oxidation against nystatin (Table IV).

The effect of dinitrophenol and of arsenate

TABLE IV. Effect of Uranyl Acetate, Sodium Arsenate, and Dinitrophenol on Metabolism of *S. cerevisiae*.

Test material*	Nystatin,† μg/ml	μl O_2 consumption 60 min.	μl O_2 consumption 120 min.	Metabolic activity ceased, min.
Control	0	585	1100	>120
	3.5	70	70	25
	5	115	115	15
Uranyl acetate, 1 × 10^{-4} M	0	304	580	>120
	3.5	350	465	120
Sodium arsenate, 2 × 10^{-2} M	0	170	345	>120
	5	200	310	110
Dinitrophenol, 2 × 10^{-4} M	0	450	940	>120
	5	310	360	45

* Uranyl acetate added 20 min. before glucose and nystatin; other test materials added with glucose and nystatin at 0 min.

† Purified preparation.

was investigated, since Halvorson *et al.*(13) reported that the former, but not the latter, inhibits uptake of amino acids into the amino acid pool of yeast. At the concentration employed by these investigators (2×10^{-4}M), dinitrophenol was relatively ineffective in reducing absorption of nystatin (inhibition 20% or less) and provided only a short protection of oxidation (Table IV). At a 2×10^{-2}M concentration, arsenate inhibited nystatin uptake ca. 60%, and this effect was not prevented by 6.7×10^{-2}M phosphate. Arsenate did not produce a significant stimulation of endogenous respiration. It reduced glucose oxidation sharply, and effectively protected the residual respiration against nystatin for the period of the test. With a 8×10^{-2}M arsenate concentration, oxidation was inhibited almost completely, whereas 4×10^{-3}M did not produce significant protection. For inhibition of nystatin uptake to occur, arsenate must be present during contact between nystatin and yeast, since cells incubated with arsenate for 30 minutes and then washed retained their ability to absorb the usual amounts of nystatin. When yeast was incubated with nystatin (5 μg/ml) until oxidation of glucose had ceased, addition of arsenate did not restore activity. Thus the action of arsenate appears to be preventive only.

Discussion. The results lend considerable support to the proposal that nystatin uptake by cells is critical for its inhibitory action(3). The protective action of the various agents for metabolism correlates well with conditions under which they absorb significant amounts of nystatin or reduce rate or extent of its absorption by yeast. The characteristic of this protection is increase in time before a sharp drop in metabolic activity occurs. This pattern was predicted from the time sequence of nystatin action illustrated in Fig. 1. However, of 4 resistant strains tested, 2 had changes in sensitivity greater than one would anticipate from the small reductions in amount of nystatin they absorbed. Thus, factors other than uptake must also be important in determining sensitivity to nystatin.

The kinetics of metabolic inhibition by nystatin are compatible with the concept that nystatin initiates a destructive action on critical cell constituents. Scholz *et al.*(14) have shown that nystatin produces a rapid decrease in amounts of glycolytic enzymes and protein extractable from the yeast cell. It is probable that these enzymes are initially present in excess, so that no effect on glycolysis is observed in our experiments until the enzymes become rate-limiting. Concentration of nystatin would determine rate of this destruction and thus the time until metabolic changes become evident.

Previous work(2) has shown that uptake of nystatin is sharply increased at acid pH. The apparent pK_a of the group concerned approximates that of the carboxyl of nystatin. The present finding that the methyl ester and N-acetyl derivatives are not absorbed to a significant extent allows one to suggest that the ionic species of nystatin absorbed by yeast is $HOOC \ldots NH_3^+$.

Summary. 1) Time sequence for inhibition of yeast respiration and glycolysis by nystatin is described and its significance is discussed. 2) A series of nystatin-resistant : sensitive pairs of *Candida* strains was examined. With various pairs, resistance of growth to nystatin was accompanied by similar resistance of metabolic activity to the agent. Resistant strains absorbed less nystatin than did sensitive organisms under same conditions, but there was no quantitative correlation between sensitivity and absorption. Reduced uptake of nystatin is probably only one of the ways by which resistance can be achieved. 3) Addition of serum albumin, zymosan, uranyl ions, arsenate, or dinitrophenol could protect yeast metabolism in varying degrees against the action of nystatin. Inhibition, once obtained, could not be reversed. The protective action of various agents approximated that expected from their effect on absorption of nystatin by yeast. 4) The methyl ester and N-acetyl derivatives of nystatin, which do not inhibit growth or metabolism of fungi, were not absorbed to a significant extent by either fresh yeast or zymosan. Since absorption occurs primarily below pH 5, the ionic species $HOOC \ldots NH_3^+$ is probably the material absorbed by yeast. 5) The results furnish additional evidence for the critical nature of up-

take of nystatin by microorganisms in determining sensitivity to its action.

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Effect of Urea on Enzyme Activity of the Choroid Plexus. (25099)

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Fremont-Smith and Forbes(1) demonstrated that urea when injected into peritoneum of the cat had a rather striking effect on reduction of intracranial pressure. They suggested its clinical application because of its lack of toxicity and its ready elimination by normal kidneys. Smythe, Smythe and Settlage(2) later found that 17% urea produced a profound and persistent drop in intracranial pressure. On this basis, Javid and Settlage(3) reported effective use of a 30% solution of urea in reducing the intracranial pressure of 21 patients. No secondary rise was found as had been the experience with hypertonic solutions of sodium chloride and dextrose. The effect of urea in reducing intracranial pressure is present despite bilateral nephrectomy in the monkey(4). The specific role of the choroid plexus in formation of cerebrospinal fluid is not understood. To gain insight into the role of this organ, the activity of 7 representative enzymes has been measured in the choroid plexus of the cat(5). This tissue was approximately one-third to one-half as active as liver and kidney and more active than most areas of the brain in specific reactions. Our findings do not allow us to conclude that this structure is responsible for production of CSF, but its anatomical and enzymatic properties strongly suggest that it may play a role in elaboration of this fluid. Since urea has a profound effect upon intracranial pressure, it became of interest to study the effect of this drug upon selected enzymatic activities of the choroid plexus.

Materials and methods. All studies were done on the cat. The animals were anesthetized with intravenous nembutal, 30 mg/kg, and the animal placed in the prone position. The cisterna magna was entered with a fine 25 gauge needle attached to polyethylene catheter connected to a pipette clamped in horizontal position and graduated in hundredths of a milliliter. Observations of the CSF flow were made every 10 minutes. After urea had been given intravenously, observations were continued for 3 hours. At end of experiment, the animal was sacrificed, and a sample of blood, a kidney, and entire brain were removed. The choroid plexus along with