TABLE II. LD Activity Units Released when 100,000 Cells Were Disrupted.

Cell line	Type of cell	LD activity, units/cc
HAF	Normal	1400
Amnion	"	768
Conjunctiva	Transformed	480
FL amnion	"	230
Liver	"	68
HEP #1	Malignant	132
HEP #2	"	142
J-111	"	140
Skiff	"	38

in the cells themselves and a comparable increase is found in the medium surrounding them. This would seem to be a condition analogous to that found clinically when fluids containing exfoliated cells are found to be the richest in the enzyme activity.

When LD activity for the cell lines derived from normal, transformed, and cancer sources, and grown in tissue culture, are compared, large amounts of activity are found in the known cancer cell lines and in the transformed lines, but little in the normal cell lines. This appears to be a reflection of the rapid growth rate, however, rather than a characteristic of the cell itself, since LD activity per cell is found to be greater in both the normal fibroblasts and amnion cells than in the other cells. How the large amount of LD activity in the fluid bathing the malignant cells accumulates is unknown, but one may speculate that it could diffuse out through the cell wall, or it may escape into the media at the time of cell division.

Summary. Normal, malignant, and transformed cell lines have been studied for variations in 3 different enzyme activities, GO-T, GP-T, and LD. Only LD activity changed during the growth period. The enzyme activity both in the cells and in the fluid bathing them was greatly increased in the malignant cell lines and in 2 transformed lines, in contrast to the normal cell lines and the conjunctiva cell line where large quantities did not appear in the media. The increase in LD activity was found to be related to the cellular growth rate rather than to be a characteristic of the cell, since the normal cells in most instances were found to contain more enzyme activity per cell than the malignant ones.

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Interactions Between Phosphate and Nystatin in *Candida stellatoidea*.* (24186)

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Nystatin, an antibiotic produced by *Streptomyces noursei*, inhibits growth of fungi but does not affect growth of bacteria and viruses. It is therefore widely used clinically against a variety of mycotic infections, particularly candidiasis(1). Neither the structure nor mode

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of action of nystatin has been elucidated. It is recognized that this antibiotic inhibits utilization of several substrates normally metabolized by yeasts and molds(2). However nystatin does not inhibit oxidation of citrate, α-ketoglutarate and glutamate. Of particular interest is the observation that nystatin inhibits net uptake of inorganic phosphate(3). This report presents data which suggest that nystatin affects the normal role of phosphate in *Candida stellatoidea*.

Methods. Stock cultures of *C. stellatoidea*† were maintained on the complete medium previously described(4). Cells for manometric studies were subcultured continuously as the yeast phase in glucose broth of the following composition: glucose, 60 g; Bacto peptone, 5 g; Bacto yeast extract, 2.5 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 1 g; $(NH_4)_2SO_4$, 2 g; de-ionized water, 1 L. The pH was adjusted to 4.5 prior to sterilization at 121°C for 12 minutes. After incubation at 30°C on a rotary shaker, the 18-hour cultures were harvested by centrifugation and washed twice with cold de-ionized water. The washed cells were then suspended in either 0.067 M phosphate or phthalate buffer which was adjusted to pH 4.5. To each Warburg vessel, 5 mg dry weight of cells were dispensed into the main chamber. Nystatin‡ was added, when indicated, as an aqueous suspension (1500 μg/ml) to the sidearm. Glucose (100 mg/flask) was placed in the second sidearm. All manometric studies were conducted anaerobically by flushing with nitrogen. Respirometers were maintained at 30°C and the shaking rate was 95 strokes/min. The nystatin resistant and the arsenate resistant substrains were obtained from the sensitive parental strain by direct selection(5). The antibiotic was added aseptically to the complete medium as an alcoholic suspension. Mutants resistant to increasing concentrations of nystatin were selected by subculturing survivors on increasing concentrations of the antibiotic. The following increments were employed: 1, 2, 3, 4, 6, 8, 12, and 16 μg/ml.

† Kindly supplied by Dr. Norman F. Conant, Duke University.

‡ Mycostatin, E. R. Squibb & Sons, N. Y.

TABLE I. Inhibitory Effects of Azide and Arsenate on Glucose Fermentation by Nystatin Sensitive and Nystatin Resistant Strains of *Candida stellatoidea*. Warburg vessels contained 5 mg cells (dry wt), 100 mg glucose, phthalate buffer pH 4.5, nitrogen atmosphere.

Inhibitor		μl carbon dioxide evolved/hr	
		Resistant strain	Sensitive strain
Sodium azide	3×10^{-3} M	64	70
	3×10^{-4} M	90	91
	3×10^{-5} M	120	124
Monobasic sodium arsenate	2×10^{-2} M	90	76
	10^{-2} M	105	84
	5×10^{-3} M	138	104
	10^{-3} M	150	123
	10^{-4} M	150	145
Control		150	150

Similarly growth from the complete medium containing 0.01 M NaH_2AsO_4 was plated on complete medium containing 0.02 M arsenate. Resistant strains were subcultured at least once in the absence of inhibitor prior to each experiment.

Results. The effect of metabolic poisons on glucose fermentation by parental and nystatin resistant strains was determined. As shown in Table I, sodium azide, at concentration of 0.003 M, reduced fermentative production of CO_2 by 50% whereas 0.00003 M azide reduced CO_2 evolution by 20%. In this instance the nystatin sensitive and resistant strains responded identically.

Arsenate likewise inhibited glucose fermentation by both the nystatin sensitive and nystatin resistant strains. Unlike azide, arsenate inhibited the parental strain more than the nystatin resistant strain. At a concentration of 0.001 M arsenate, CO_2 production by the nystatin resistant strain was not affected, whereas under the same conditions CO_2 production by the parental strain was reduced 15%.

The inhibitory effect of 0.01 M arsenate on glucose fermentation by the nystatin sensitive parental strain was completely reversed by 0.067 M phosphate buffer (Table II). This is consistent with the concept that arsenate inhibits oxidative phosphorylation of 3-phosphoglyceraldehyde in the Embden-Meyerhof scheme. Additional data, not presented here, plotted according to Lineweaver

TABLE II. Reversal by Phosphate of Inhibitory Effects of Arsenate and Nystatin on Glucose Fermentation by the Parental Strain. Warburg vessels contained 5 mg cells (dry wt), 100 mg glucose, either phthalate buffer or phosphate buffer pH 4.5, nitrogen atmosphere.

Inhibitor	μl CO_2/hr	
Monobasic sodium arsenate	no phosphate	.067 M phosphate
2×10^{-2} M	88	134
10^{-2} M	105	169
5×10^{-3} M	145	202
Control	150	150
Nystatin	no phosphate	.5 M phosphate
60 μg/ml	96	107
40 "	110	136
20 "	117	150
Control	150	150

and Burk, confirmed the competitive nature of the arsenate-phosphate interaction.

These findings suggested that inhibition of glucose fermentation caused by nystatin might be reversed by the presence of phosphate. Although 0.067 M phosphate was not effective in this respect, addition of 0.5 M phosphate completely canceled the 20% inhibition produced by 20 μg nystatin/ml.

As shown previously, the nystatin resistant strain was less susceptible to arsenate than its nystatin sensitive counterpart. Moreover inhibition of glucose fermentation produced by arsenate and nystatin could be reversed by appropriate concentrations of phosphate. On the basis of these findings it was postulated that an arsenate resistant mutant might have increased resistance to nystatin. Indeed experiments confirmed that glucose fermentation by the arsenate sensitive parental strain was reduced 20% by 20 μg nystatin ml whereas the arsenate resistant strain was not affected by this concentration of nystatin (Table III).

The significance of the manometric results, as correlated with the fungistatic property of the antibiotic, was determined by growth experiments. Phosphate, added to complete medium to a maximal concentration of 0.5 M, did not reduce the fungistatic activity of nystatin (4 μg/ml). However growth experiments confirmed that the nystatin resistant strain was less susceptible to arsenate than the parental strain. Moreover the arsenate resistant strain was more resistant to nystatin than its arsenate sensitive counterpart.

Discussion. One of the pertinent findings of this study was that a greater concentration of nystatin was required to inhibit growth of *Candida stellatoidea* in glucose broth than in the complete broth. This is consistent with the report that glucose and peptone reverse nystatin inhibition(6). It is of interest that growth of the *C. stellatoidea* strain we used was inhibited by as little as 1.3 μg nystatin/ml in the complete medium. After several subcultures in the presence of increasing concentrations of nystatin, a strain resistant to 16 μg/ml was obtained. Because it is reportedly difficult to isolate mutants resistant to nystatin, it is noteworthy that the resistant strain described here developed such a high degree of resistance(7).

Our findings show that resistance to nystatin was accompanied by resistance to arsenate. Similarly selected resistance to arsenate was associated with increased resistance to nystatin. Such results suggest that nystatin may affect the oxidative phosphorylation of 3-phosphoglyceraldehyde to 1,3-diphosphoglyceric acid. This is consistent with the recognized mode of action of this inhibitor. Nystatin could therefore exert its inhibitory effect 1) by complexing with 3-phosphoglyceraldehyde, 2) by binding inorganic phosphate essential for oxidation, 3) by interfering with diphosphopyridine nucleotide, or 4) by inactivating the enzyme itself. In as much as inhibition of glucose fermentation by nystatin is reversed by phosphate, it is conceivable that nystatin may act by binding inorganic phosphate. It is pertinent in this

TABLE III. Inhibitory Effect of Nystatin on Glucose Fermentation by Parental and Arsenate Resistant Strains of *Candida stellatoidea*. Warburg vessels contained 5 mg cells (dry wt), 100 mg glucose, phosphate buffer pH 4.5, nitrogen atmosphere.

Nystatin	μl CO_2/hr	
	Sensitive strain	Resistant strain
100 μg/ml	18	80
60 "	79	113
20 "	120	158
Control	150	150

regard to note that nystatin activity is reduced by cysteine and that cysteine reactivates triosephosphate dehydrogenase which has been inactivated by oxidation. These latter observations may have a causal relationship.

The manometric studies reported here have been concerned only with fermentation, in spite of the fact that it has been previously shown that respiratory reactions are also affected. Therefore the effects of nystatin reported herein are but one of several possible manifestations. It is premature on the basis of these data to assert that the primary mode of action of nystatin concerns oxidative phosphorylation. Certainly this is an important site and the correlation between manometric findings and growth studies constitutes circumstantial evidence that this may be the case.

Summary. Glucose fermentation by *Candida stellatoidea* was inhibited by sodium arsenate and by the fungistatic antibiotic, nystatin. Mutants selected for resistance to one inhibitor simultaneously developed increased resistance to the other inhibitor. The inhibitory effects of arsenate and nystatin on glucose fermentation by the sensitive parental strain were reversed by appropriate concentrations of phosphate.

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Studies on Erythropoiesis IX. Mechanism of Decreased Erythropoiesis In Experimental Polycythemia. (24187)

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That increased erythropoiesis following production of acute anemia is mediated on a hormonal basis was first suggested by Carnot and Deflandre(1). In recent years, numerous investigators have demonstrated the appearance of a humoral erythropoietic-stimulating factor in anemic animals(2-9). Fried, Plzak, Jacobson, and Goldwasser(10) have presented evidence supporting the hypothesis that the rate of erythropoiesis is determined by the amount of erythropoietin in plasma at all times. It has long been known that experimentally-induced polycythemia depresses erythropoiesis(11-15). That decreased concentrations of erythropoietin are responsible for depression of erythropoiesis in transfusion-induced polycythemia was first suggested by Fried *et al.*(10) and Jacobson *et al.*(16). Demonstration of decreased concentrations of erythropoietin in plasma of transfusion-induced plethoric animals and human beings is as important in establishing validity of the concept of a humoral control of erythropoiesis as is demonstration of increased erythropoietin concentrations in plasma obtained from anemic donors. In this paper, the decline in reticulocyte count and incorporation of Fe^{59} into newly-formed red cells of polycythemic rats, and degree of decline in relation to severity of polycythemia are reported. In addition, response of these polycythemic animals to anemic plasma and cobaltous chloride is described.

Methods and materials. Rats of the Sprague-Dawley strain were used in this study. Animals in last experiment were hypophysectomized by Hormone Assay Laboratories of Chicago. Blood was removed from donor animals by cardiac puncture, using heparin as anticoagulant. Intravenous trans-