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Inhibition of Growth, Synthesis, and Permeability in *Neurospora crassa* by Phenethyl Alcohol

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ABSTRACT

LESTER, GABRIEL (Reed College, Portland, Ore.). Inhibition of growth, synthesis, and permeability in *Neurospora crassa* by phenethyl alcohol. J. Bacteriol. **90**: 29–37. 1965.—Inhibition of the growth of *Neurospora crassa* in still culture was detected at 0.05% and was complete at a level of 0.2% phenethyl alcohol (PEA). Benzyl alcohol was less inhibitory, and 3-phenyl-1-propanol and phenol were more inhibitory, than PEA; benzylamine and phenethylamine were less inhibitory than the analogous hydroxylated compounds. Inhibition by PEA was not reversed by synthetic mixtures of purines and pyrimidines or vitamins, or by casein digests, yeast extract, or nutrient broth. The germination of conidia was inhibited by PEA, but after an exposure of 8.5 hr no loss of viability was observed. The addition of PEA to growing shake cultures caused a simultaneous inhibition of growth and of the syntheses of ribonucleic and deoxyribonucleic acids and protein; the relationships of these compounds to mycelial dry weight and to one another were constant in growing mycelia, and PEA did not significantly affect these relationships. PEA partially inhibited the uptake of glucose, but severely restricted the accumulation of L-leucine, L-tryptophan, or α-aminoisobutyric acid in germinated conidia. The efflux of α-aminoisobutyric acid from germinated conidia was somewhat enhanced by PEA, but this effect was not so pronounced as the (complete) inhibition of α-aminoisobutyric acid accumulation by PEA. It is suggested that PEA affects primarily the initial influx of α-aminoisobutyric acid rather than the subsequent retention of α-aminoisobutyric acid.

The discovery (Berrah and Konetzka, 1962) of a selective inhibition of deoxyribonucleic acid (DNA) synthesis in *Escherichia coli* by phenethyl alcohol (PEA) has led to the use of this compound in elucidating the role of DNA in various biological phenomena, e.g., in studies of the replication of bacteriophage (Konetzka and Berrah, 1962; Folsome, 1963; Nonoyama and Ikeda, 1964) and of the transfer of genetic material during the conjugation of bacteria (Bouck and Adelberg, 1963; Roeser and Konetzka, 1964). PEA inhibition of sporulation and germination in *Bacillus megaterium* (Slepecky, 1963) and of the multiplication of herpes simplex virus (Roizman, 1963) has also been observed. Relatively little work has been done on the mechanism of action of PEA, although it has been indicated (Bouck and Adelberg, 1963) that PEA might affect processes other than DNA replication, and more recently it has been suggested (Treick and Konetzka, 1964) that the primary site of PEA action in *E. coli* might be on the site(s) of the bacterial membranes which possibly serve as foci for the initiation of DNA replication (Jacob, Brenner, and Cuzin, 1963).

The obvious utility of PEA in examining various aspects of viral and bacterial processes raises the possibility of its potential application to studies with higher, eucaryotic organisms. To this end, the effects of PEA on the growth and metabolism of *Neurospora crassa* have been examined.

MATERIALS AND METHODS

Organisms. N. crassa strain 74A was the subject of these studies, except for one experiment (see Table 1). Stock cultures were maintained on H. J. Vogel's synthetic medium N (Lester and Hechter, 1961) containing 1.0% sucrose and 1.5% agar.

Culture conditions. For still cultures, Fries medium (Beadle and Tatum, 1945) containing 2.0% sucrose was used unless otherwise indicated. All still cultures were prepared in 125-ml Erlenmeyer flasks containing 20 ml of medium. A drop of a light suspension of conidia was used to inoculate each flask, and the flasks were incubated at 30 C for 72 hr unless otherwise indicated.

Fries medium containing 1.0% sucrose was used for shake cultures. These cultures were started with an inoculum of 2.5×10^7 conidia per milliliter, and they were incubated at 30 C on a rotary

shaker describing a 1.9-cm circle) at 200 oscillations per min. Under these conditions, a disperse growth, without clumping, was obtained, and the culture could be handled with a pipette with a tip bore 2 to 2.5 mm in diameter. The production of conidia for massive inocula and for the preparation of germinated conidia used in uptake studies were described previously (Lester, 1961).

For the estimation of dry weights, pads from still cultures and measured portions of shake cultures were harvested on a filter with suction, rinsed with water, and dried for 18 to 24 hr at 100 C. The volume of samples taken from shake cultures was of a size sufficient to yield at least 50 mg of dried cells.

Estimation of the ribonucleic acid (RNA), the DNA, and the protein content of cells grown in shake culture. Measured samples of shake cultures were taken in duplicate, filtered, and washed with ice water; one of the samples was dried for a weight determination, and the other sample was frozen and stored at −20 C until all the samples required in an experiment had been taken (and the dry weight of the comparable sample was determined). The general procedure (Minagawa, Wagner, and Strauss, 1959) for the estimation of these cellular components involved an extraction of materials of low molecular weight, which appeared to interfere with the RNA assay. This was followed by a stepwise separation of the other components: a mild digestion of RNA with cold perchloric acid, digestion of DNA with hot perchloric acid, and the solubilization of protein in the remaining material with alkali.

The frozen samples of mycelia were thawed and extracted successively with 10 ml of 10% cold trichloroacetic acid, 10 ml of 95% ethyl alcohol, 10 ml of 100% ethyl alcohol, 10 ml of ethyl alcohol-ether (3:1) for 5 min at 60 C, and, finally, with 10 ml of ether. Each extract was separated by centrifugation; the supernatant liquid was discarded, and the extracted material obtained was dried with gentle warming.

The extracted material was suspended in cold 0.5 N perchloric acid, 1.0 ml for each 10 mg (dry weight) of cells originally present, and the suspension was held at 4 C for 48 hr with occasional mixing. The suspension was centrifuged, the supernatant liquid was decanted, and the sediment was suspended in 2 to 3 ml of cold 0.5 N perchloric acid; the suspension was centrifuged, and the supernatant fractions were combined. The supernatant fraction was assayed for RNA by the method of Brown (1946), with yeast RNA (Nutritional Biochemicals Corp., Cleveland, Ohio) as a reference standard. Neither DNA nor protein could be detected in this extract, and the values for RNA obtained by the chemical assay were the same as those obtained spectrophotometrically with the same reference standard. The absorption curves for perchloric acid digests of the cells and the reference standard were very similar.

The solid material remaining after the extraction of RNA was suspended in 5 ml of 0.5 N perchloric acid and heated for 60 min at 85 C, then cooled and centrifuged. The pellet was taken up in 2 to 3 ml of 0.5 N perchloric acid and centrifuged; the supernatant fractions were combined. The supernatant fraction was assayed for DNA by the method of Burton (1956), with highly polymerized salmon sperm DNA (Mann Research Laboratories, New York, N.Y.) as a reference standard. Neither RNA nor protein could be detected in this extract. The values obtained for DNA were the same as those obtained with hot perchloric acid extracts of fresh cells, which indicated that no degradation of DNA had occurred in the procedure used to extract RNA.

The solid material remaining after the extraction of RNA and DNA was suspended in 5 ml of 1.0 N NaOH and held at room temperature with occasional mixing. Then 5 ml of water were added and mixed, and the suspension was centrifuged. The supernatant liquid was assayed for protein by the method of Lowry et al. (1951), with bovine-plasma fraction IV (Armour and Co., Chicago, Ill.) as a reference standard. (Since a large dilution was required for this assay, the alkaline suspension could be used without prior centrifugation.)

The procedure described above gave very reproducible results with replicate samples.

Uptake of glucose and amino acids. The general methods used to examine the uptake of these compounds were described elsewhere (Lester, Stone, and Hechter, 1958), and they will be summarized only briefly here. The system used consisted of a suspension of germinated conidia [4 to 6 mg (dry weight) per ml] in 0.02 M phosphate buffer (*p*H 5.8) and 0.05 M sodium chloride; the suspensions were incubated at 30 C with agitation in a reciprocating water-bath shaker. The desired compound was added, samples were taken at intervals, and the cells were removed by filtration. The filtrates were analyzed for the compound being examined, and the difference between the initial and subsequent values represented the uptake which had occurred.

Glucose was assayed with Glucostat (Worthington Biochemical Corp., Freehold, N. J.). The uptake of α-aminoisobutyric acid-*1*-C^{14} (AIB) and L-leucine-*1*-C^{14} was estimated from the disappearance of radioactivity from the buffer; L-tryptophan was measured by bioassay with *E. coli* strain T-3, A-11 (obtained from C. Yanofsky), which has a double block in tryptophan biosynthesis. The basal medium used for the tryptophan assay was medium E (Vogel and Bonner, 1956) containing 0.25% glucose and 0.05% Casamino Acids. Under the above conditions, the glucose taken up is completely metabolized (no free glucose can be detected in the cells); part of the tryptophan and leucine taken up is metabolized, although there is still an intracellular accumulation against a concentration gradient; and none of the AIB taken up is metabolized (Lester, *unpublished data*).

Chemicals. The chemicals used in these studies were obtained from commercial sources and used without further purification or analysis. The radioactive amino acids were obtained from New

England Nuclear Corp., Boston, Mass.; benzyl alcohol, from Fisher Scientific Co., Pittsburgh, Pa.; and PEA, phenethylamine, benzylamine, and 3-phenyl-1-propanol, from Eastman Kodak Co., Rochester, N. Y. PEA and its related compounds were sterilized by filtration.

RESULTS

Effect of PEA on the growth of certain molds. Table 1 shows that PEA can inhibit the growth of several species of molds. Because of differences in the growth rates of these species and in the culture conditions, an evaluation of relative sensitivities among these species cannot be rigorously made without more refined tests. *Penicillium notatum* appeared to be the most sensitive and *Rhizopus nigricans* the least sensitive to PEA under the conditions employed. However, it is of more importance that all the species examined were inhibited by a very similar range of concentrations of PEA, and that these concentrations were similar to those which inhibited the growth of several diverse bacterial species (Berrah and Konetzka, 1962). All the subsequent studies were carried out with *N. crassa.*

Specificity of PEA as an inhibitor. Several compounds related to PEA were examined for their effect on the growth of *N. crassa* in still culture. Table 2 shows that the inhibitory effect of PEA is shared by other substituted benzene compounds, but that the intensity of inhibition depends on the nature of the substitution. The strong inhibition by phenol was markedly reduced by the interposition of a methylene group between the benzene ring and the hydroxyl group (benzyl alcohol). However, with the increase in the size of the interposed aliphatic chain, an increase in inhibitory activity was observed, and the propanol derivative was almost as inhibitory as phenol. Substitution of the hydroxyl group by an amino group also resulted in a decrease in inhibitory effect.

Effect of various compounds on the inhibition of growth by PEA. The inhibition of growth in still culture by PEA could not be reversed by the addition to the medium of materials such as yeast extract (Difco) or a casein hydrolysate (Sheffield N-Z-case), nor by synthetic mixtures of purines and pyrimidines (adenine, guanine, cytosine, uracil, thymine) or vitamins (thiamine, riboflavine, pyridoxine, niacinamide). These ob-

TABLE 1. *Effect of PEA on the growth of certain molds*

Organism	Incubation		PEA	Growth*	Inhibition
	Temp	Time			
	C	*hr*	%†	*mg*	%
Aspergillus niger	30	96	0	227	—
			0.1	127	44
			0.2	0	100
			0.4	0	100
Penicillium notatum	22	96	0	124	—
			0.1	7	94
			0.2	0	100
			0.4	0	100
Rhizopus nigricans	22	96	0	14	—
			0.1	10.5	25
			0.2	6.4	54
			0.4	0	100
Neurospora crassa	30	72	0	98	—
			0.1	34	65
			0.2	0	100
			0.4	0	100

* Vogel's medium N, supplemented with sucrose (2%, w/v) and Sheffield N-Z-case (0.1%, w/v); 20 ml per 125-ml Erlenmeyer flask. The values given are the average dry weight of the mycelial pads from duplicate flasks.

† By volume.

TABLE 2. *Effect of PEA and related compounds on the growth of Neurospora crassa*

Compound	Concn	Growth*	Inhibition
	%†	*mg*	%
None	—	83	—
Phenol	0.05	13	83
	0.15	0	100
	0.30	0	100
Benzyl alcohol	0.05	52	36
	0.15	31	63
	0.30	3	96
Phenethyl alcohol	0.05	68	18
	0.15	28	66
	0.30	0	100
3-Phenyl-1-propanol	0.05	46	64
	0.15	0	100
	0.30	0	100
Benzylamine	0.05	76	7
	0.15	67	19
	0.30	53	35
Phenethylamine	0.05	96	−16
	0.15	82	0
	0.30	44	67

* The values given are the average dry weight of the mycelial pads from duplicate flasks.

† By volume.

TABLE 3. *Effect of PEA concentration on growth and the synthesis of RNA, DNA, and protein in Neurospora crassa*

Incubation time	PEA at 5 hr	Growth	Amt (μg)/ml of culture			Amt (μg)mg of dry wt		
			RNA	DNA	Protein	RNA	DNA	Protein
hr	%*	*mg/ml*†						
0	—	0.52	37	2.1	165	71	4.1	318
3	—	0.92	84	3.1	319	91	3.4	346
5	—	1.38	122	4.6	460	89	3.3	334
9	—	3.37	276	11.5	1,280	82	3.4	380
9	0.1	2.73	216	8.6	1,040	79	3.2	380
9	0.2	2.16	150	6.7	780	70	3.1	360
9	0.3	1.67	134	5.6	655	80	3.3	392

* By volume.
† Dry weight.

servations are consistent with the observations (Berrah and Konetzka, 1962) of the inhibition by PEA of an increase of viable bacteria in a relatively rich medium. However, with bacteria, increases in mass or turbidity were observed, and additions of yeast extract resulted in further increases in turbidity. With *N. crassa* the inhibition of growth appears to be complete and irreversible by various nutrients. In similar experiments with lower concentrations of PEA (0.05 and 0.1%), which do not cause a complete inhibition of growth, no reversal or sparing of the inhibition by various compounds was observed.

Effect of PEA on the viability of conidia. Conidia were incubated in Fries medium containing 2% sucrose (w/v) and 0.3% PEA (v/v). Incubation was at 30 C without agitation, except at the time of sampling when the suspension was shaken vigorously. Samples were taken at intervals, and appropriate dilutions were plated in Fries medium containing 0.1% sucrose, 1.0% sorbose, and 1.5% agar (all w/v); portions of the diluted suspensions were mixed with 4 ml of molten medium and poured on a 15-ml solid base layer of the same medium. Viability of conidia was estimated from the plate counts of colonies which appeared after incubation at 30 C for 72 hr. Seven samples were taken in duplicate during a period of 8.5 hr, and the corresponding plate counts indicated that no loss of viability had occurred. This result coincides with the observation of Berrah and Konetzka (1962) that PEA is bacteriostatic rather than bactericidal.

The completeness of growth inhibition by PEA was further evidenced by microscopic examination of the conidial suspension during the course of exposure to PEA. No germination of conidia occurred during this period, whereas in the absence of PEA virtually all the conidia germinated. Therefore, it can be concluded that, in contradistinction to bacteria, the growth of *N. crassa* is completely inhibited by PEA.

Effect of PEA on the synthesis of macromolecules in N. crassa. The effect of PEA on growth and the synthesis of RNA, DNA, and protein was examined in shake cultures (Table 3). One large culture was started with a heavy inoculum of conidia, and samples were taken at 0, 3, and 5 hr. At 5 hr, the remaining culture was divided into four portions to which PEA was added to concentrations of 0, 0.1, 0.2, and 0.3% (v/v). After another 4 hr of incubation, the cultures were sampled again, and the dry weights, RNA, DNA, and protein of the samples were determined. In effect, then, these parameters of growth and biosynthetic activity were estimated during a 4-hr incubation period in relation to the concentration of PEA. In the absence of PEA, an exponential increase in cell mass occurred during the 9-hr incubation period, but during the last 4 hr of this period, in the presence of increasing levels of PEA, the accretion of cell mass was correspondingly decreased. A similar pattern was observed for RNA, DNA, and protein; the relationship of these components to cell mass remained constant. Thus, no major selective effect of PEA on the synthesis of RNA, DNA, or protein could be observed. Indeed, the only significant change in the relationships between these macromolecules occurred during the first 3 hr of growth, which represented the germination of conidia.

Table 4 shows the results obtained by a similar experiment wherein the effect of one level of PEA (0.25%) was examined at various times during the incubation of a shake culture. During a 6-hr period after the addition of PEA, no significant change in cell mass, RNA, DNA, or protein was observed. The relationships among these macromolecular components and cell mass also remained constant, whether the culture was

TABLE 4. *Effect of PEA on the synthesis of RNA, DNA, and protein during the growth of Neurospora crassa*

Incubation time	PEA (0.25%, v/v)	Growth	Amt (μg)/ml of culture			Amt (μg)/ml of dry wt		
			RNA	DNA	Protein	RNA	DNA	Protein
hr		*mg/ml**						
0	—	0.54	37	1.4	154	69	2.6	286
3	—	0.91	75	2.0	296	82	2.2	326
6	—	1.76	139	3.6	567	79	2.1	323
9	—	3.25	250	6.8	1,160	77	2.1	357
12	—	5.82	428	11.6	2,080	74	2.0	357
6	+	1.76	136	3.6	583	78	2.1	343
9	+	1.79	126	3.6	610	71	2.0	340
12	+	1.80	122	3.6	630	68	2.1	350

* Dry weight.

TABLE 5. *Effects of various nutrients on the inhibition by PEA of the synthesis of RNA, DNA, and protein in Neurospora crassa*

Incubation time	Addition at 5 hr*	Growth	Amt (μg)/ml of culture			Amt (μg)/mg of dry wt		
			RNA	DNA	Protein	RNA	DNA	Protein
hr		*mg/ml*†						
5	—	2.29	206	8.1	823	90	3.5	360
9	None	4.06	333	12.9	1,515	82	3.2	372
9	PEA	2.36	205	8.5	948	87	3.6	400
9	PEA + A	2.57	214	8.0	950	83	3.1	370
9	PEA + B	2.58	211	8.7	994	82	3.4	385
9	PEA + C	2.59	232	8.5	1,070	90	3.3	412
9	PEA + D	2.59	223	8.6	1,090	86	3.3	420

* PEA, 0.25% (v/v), and the following at 5 mg/ml: A, Difco nutrient broth; B, Difco yeast extract; C, Difco Casamino Acids; D, Sheffield N-Z-case.

† Dry weight.

incubated in the absence or the presence of PEA. Here again, no preferential effect of PEA on the synthesis of the compounds examined could be discerned. Moreover, the data show that PEA not only prevents the synthesis of RNA, DNA, and protein, but also causes no significant degradation of these cellular components. As in the previous experiment, the major changes observed were those occurring during the period when the conidia used as an inoculum were germinating.

Similar values for cell mass, RNA, and protein were obtained in both experiments, but consistently different values were found for DNA. The basis for this difference is not known, but it might be related to the differences in the age of the conidia used as inocula. The values for DNA in conidia presented here are lower than those obtained by others (Schulman and Bonner, 1962; Minagawa et al., 1959), but after germination (in hyphae) they are similar to those reported by Schulman and Bonner (1962). The values obtained for RNA and protein lie between those reported by these investigators; these differences might reflect different cultural conditions.

The ability of various nutrient materials to reverse the inhibitory action of PEA on the synthesis of macromolecules was also examined in shake cultures, under conditions similar to those above, where active growth and macromolecular syntheses were occurring at the time of addition of PEA. These nutrient materials were added simultaneously with PEA when the cultures were 5 hr old. Table 5 shows that none of these materials had any significant effect on the inhibitory effect of PEA on the synthesis of RNA, DNA, or protein. The small increases in these compounds in the culture were consistent with the small increases in dry weight which occurred, and no selective reversal of the PEA inhibition was observed.

It is clear that PEA inhibits the synthesis of macromolecules in *N. crassa*, but, since macromolecules represent a summation of many metabolic activities, there are many possible sites for the inhibitory action of PEA. The syntheses of RNA, DNA, and protein are sufficiently different mechanistically, and the action of PEA is so lacking in selectivity at a macromolecular level,

TABLE 6. *Effect of PEA on the uptake of amino acids by Neurospora crassa*

Amino acid	Initial concn	Time	Uptake*	
			−PEA	+PEA
	μmoles/ml	*min*		
L-Leucine	2.0	40	70	Nil
		80	160	13
		120	235	24
L-Tryptophan	1.0	40	49	11
		80	78	19
		120	105	26
AIB	0.5	40	18	Nil
		80	50	Nil
		120	79	Nil

* Results are expressed as millimicromoles per milligram (dry weight). PEA was at 0.25% (v/v). The initial concentration of germinated conidia was 4.6 mg (dry weight) per ml.

as to tentatively preclude a site of action for PEA in the unique aspects of macromolecular syntheses. Also, the inability of the monomeric components of the macromolecules examined to reverse the action of PEA (Table 6) suggests that the inhibition observed does not involve biosynthetic activities which are unique to the formation of these smaller molecules. In view of the apparently general action of PEA, it seems likely that its site(s) of action would involve metabolic activities of general importance for many cellular processes, such as cellular energetics or permeability. The experiments which follow represent preliminary investigations of these possibilities.

Effect of PEA on the uptake of glucose and certain amino acids. Figure 1 shows that a concentration of PEA which completely inhibits growth and the synthesis of RNA, DNA, and protein reduces the rate of glucose uptake by about 45%. This constant inhibition and the fact that no significant amount of free glucose could be detected intracellularly in either the absence or the presence of PEA suggest that all the glucose entering the cells in either case was metabolized. These observations also suggest that PEA does not grossly affect the metabolism of glucose, but rather affects the processes involved in the entry of glucose into the cells; otherwise, unless both events were inhibited, glucose might be expected to accumulate intracellularly.

The uptake of certain amino acids also is adversely affected by PEA. Table 6 shows that PEA markedly reduces the uptake of L-leucine and L-tryptophan, and completely inhibits the uptake of AIB. The effect of PEA on AIB uptake is of particular interest, since AIB is not metabolized by *N. crassa*; therefore, the inhibition observed must be ascribed solely to an interference by PEA with the process(es) involved in the accumulation of AIB by *N. crassa*. It may be assumed that PEA acts similarly in inhibiting the uptake of leucine and tryptophan, because the metabolism of these compounds could account for only a small part of the observed uptake (Lester, *unpublished data*).

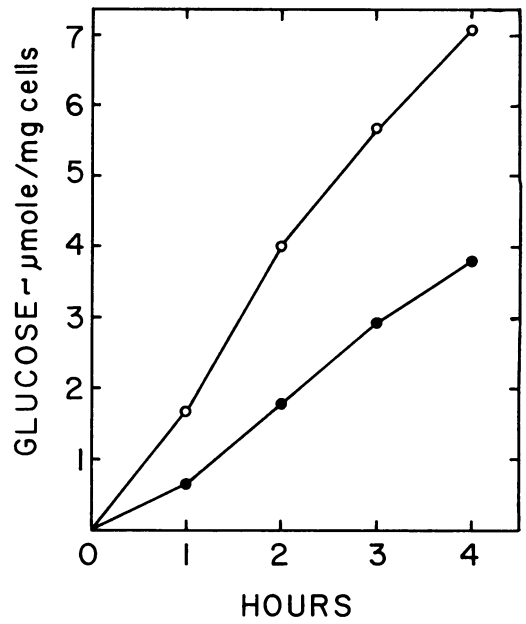

FIG. 1. *Uptake of glucose by Neurospora crassa in the absence (○) and presence (●) of PEA (0.25%, v/v). The initial concentration of glucose was 56 μmoles/ml; the concentration of germinated conidia was 5.8 mg (dry weight) per ml.*

Effect of PEA on the retention of AIB by N. crassa. It has been found (Lester and Hechter, 1958, 1959) that the uptake of potassium or rubidium by *N. crassa* represents two dissociable processes, one being the entry of these ions and the other their intracellular accumulation against a concentration gradient. Thus, it could be shown that the rate of efflux of these ions was much slower than their rate of entry; a similar situation obtains in the uptake of lactose by *N. crassa* (Lester, Azzena, and Hechter, 1962). Since the uptake of amino acids might represent a similar two-component system, it was of interest to examine the influence of PEA on the retention of AIB by *N. crassa*.

In the experiment shown in Fig. 2, germinated conidia were incubated in buffer in duplicate flasks with AIB for 2 hr; this incubation resulted

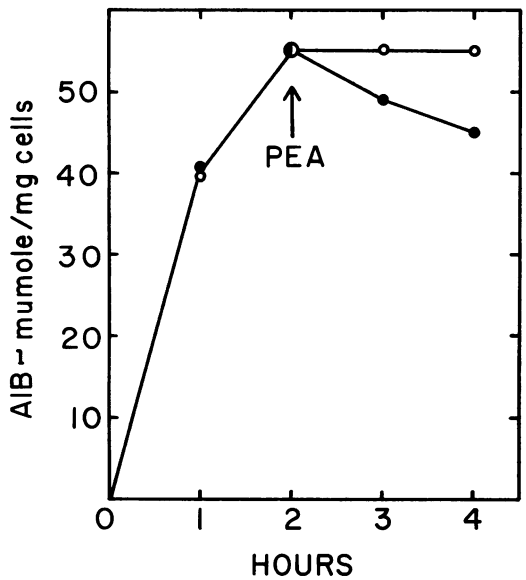

FIG. 2. *Uptake of AIB by Neurospora crassa, and the subsequent retention of AIB in the absence* (○) *and presence* (●) *of PEA (0.25%, v/v). The initial concentration of AIB was 300 mμmoles/ml; the concentration of germinated conidia was 5.4 mg (dry weight) per ml.*

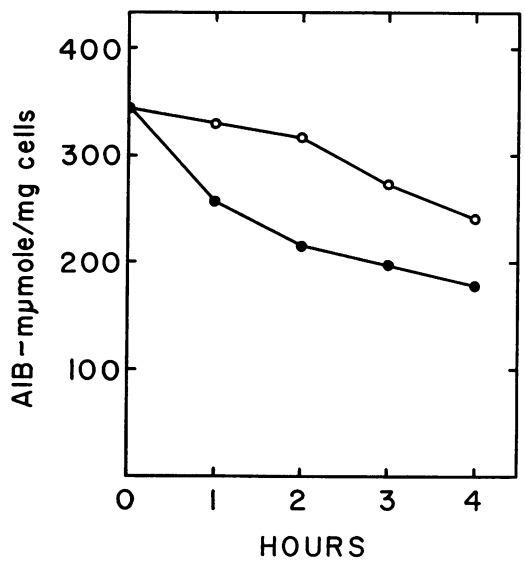

FIG. 3. *Retention of AIB by Neurospora crassa in the absence* (○) *and presence* (●) *of PEA (0.25%, v/v). The concentration of germinated conidia was 4.1 mg (dry weight) per ml.*

in the disappearance of almost all of the AIB from the suspending buffer and its appearance in the cells. At this time, PEA was added to one flask, and samples were taken from both flasks during an additional 2-hr incubation period. Cell-free filtrates of these samples were analyzed for AIB to determine its efflux from the cells. Figure 2 shows that in the absence of PEA no significant efflux of AIB occurred, whereas in the presence of PEA an efflux of AIB was observed. It should be noted that the efflux of AIB occurred at a much lower rate than its uptake.

An examination of the effect of PEA on the retention of AIB was carried out under conditions where the capacity of germinated conidia for AIB was more nearly saturated than in the preceding experiment. Conidia were germinated in a medium containing 1.5 μmoles of AIB per ml, harvested, and washed three times with buffer; an uptake of 1.3 μmoles of AIB per ml was observed, and the washed germinated conidia contained 345 mμmoles of AIB per mg (dry weight). No significant loss of AIB occurred during the washing of the cells. Duplicate portions of germinated conidia were incubated in the absence and presence of PEA. Samples were taken at intervals, and cell-free filtrates of these samples were analyzed for AIB. Figure 3 shows that during the first 2 hr of incubation an efflux of AIB occurred in both cases, but more rapidly in the presence of PEA. Subsequently, a similar rate of efflux of AIB occurred in the absence and presence of PEA. Thus, it appears that the inhibition of AIB uptake by PEA is not due primarily to an action of PEA on the retention of accumulated AIB, but rather is due to its interference with the entry of AIB into *N. crassa*.

DISCUSSION

These studies have shown that PEA inhibits the growth of *N. crassa* at concentrations similar to those which inhibit the growth of several bacterial species (Berrah and Konetzka, 1962). On a molecular level, the action of PEA on *N crassa* partially resembles its effect on *E. coli* in its interference with the synthesis of DNA. However, in *E. coli* the PEA appears to act selectively, exerting a preferential inhibition of DNA synthesis, whereas in *N. crassa* PEA inhibits to an equal extent the syntheses of RNA, DNA, and protein. Further, in *E. coli* PEA appears to inhibit the initiation of a cycle of DNA replication rather than the completion of a cycle already underway (Treick and Konetzka, 1964); in *N. crassa* PEA appears to inhibit DNA synthesis immediately. At the present time, without a definitive understanding of the exact mechanism of PEA action in either organism, it is difficult to assess precisely the basis for the differences observed. Thus, it is possible that (i) different

sites of PEA action are involved, (ii) similar sites are affected with different secondary consequences, or (iii) there is a close qualitative correspondence in both the primary and secondary effects of PEA in *E. coli* and *N. crassa*, but with different quantitative expressions. However, the present studies do afford an explanation for the inhibition of the growth of *N. crassa* by PEA, and this might bear some relationship to the effect of PEA on *E. coli*.

The uniform inhibition of growth and the varied syntheses of RNA, DNA, and protein in *N. crassa*, and the ineffectuality of various nutrients of low molecular weight in reversing these inhibitions, suggest that PEA acts indiscriminately on many processes, or that its unique site(s) of action is of general consequence for many cellular activities. Two possibilities related to the latter hypothesis have been considered: (i) cellular energetics and (ii) permeability. The interference of either of these types of cellular activity would have far-reaching secondary consequences for many other cellular processes. The first possibility has not been ruled out, and the effect of PEA on energetic processes, such as oxidative phosphorylation, should be examined. However, the fact that the gross metabolism of glucose is not inhibited by PEA to nearly the same extent as growth or macromolecular syntheses decreases the likelihood that energy metabolism is a primary site of PEA action. On the other hand, the degree of inhibition of the uptake of amino acids by PEA corresponds to its inhibition of other processes examined. The inhibition of uptake by PEA appears to be directed toward blocking the influx of amino acids rather than accelerating their efflux. This means, then, that the opportunity for the metabolism of such compounds would be severely restricted or not afforded at all by the presence of PEA. It should be noted, however, that some of the present studies were carried out in simple synthetic media, where the parameters examined were not dependent on exogenous organic materials other that a simple carbon source and biotin. Thus, the cogency of an explanation of PEA inhibition based on interference with permeability processes will depend on the demonstration that PEA inhibits the entry of normal constituents of the medium, such as inorganic ions; studies bearing on this possibility have not been made.

A relationship of the above considerations to the action of PEA on *E. coli* can be made on the grounds (i) that experiments demonstrating a selective action of PEA on DNA synthesis were carried out in media containing amino acids and other organic materials and (ii) that, although the action of PEA was selective, it was not exclusively restricted to DNA synthesis, and inhibitions of the synthesis of RNA and protein also were observed (Berrah and Konetzka, 1962; Treick and Konetzka, 1964). Thus, the possibility of an effect of PEA on permeability processes in *E. coli* should be examined. More importantly, perhaps, for developing a unifying basis for the (at least quantitatively) diverse action of PEA on *N. crassa* and *E. coli* is the question of the exact site of PEA action, which, for *N. crassa* particularly, raises the corollary question of the material basis for permeability phenomena. It is reasonable to assume that cellular membranes are involved in permeability processes, and this assumption leads to the speculation that a site of action of PEA on permeability is the cell membrane. This, then, would lead to a convergence with the view expressed by Treick and Konetzka (1964) that the selective inhibition of DNA synthesis in *E. coli* might be due to an alteration of the cell membrane by PEA so that it could not function as the proposed initiation point (Jacob et al., 1963) for the replication of the chromosome of *E. coli*. Thus, the studies presented here suggest that PEA might have a common primary action in *E. coli* and *N. crassa*, with quantitatively differing secondary consequences, which might in part reflect the dissimilarity of these organisms in terms of their procaryotic and eucaryotic characteristics. These studies also point to a potential use of PEA in the investigation of permeability processes and other membrane-based biological phenomena in fungi as well as in bacteria.

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