Chemical Induction of Colonial Paramorphs in *Neurospora* and *Syncephalastrum*[1]

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GENETIC ALTERATION in fungi has been found to give rise to two main classes of mutant strains. Most thoroughly investigated have been the "biochemical" types of mutants, in which specific defective biosyntheses have been demonstrated (*3, 4*). The other class includes the "morphological" mutant types, which differ from the wild type in growth habit, and which in general cannot be restored to normal by varying the physical or chemical environment of the mutant strain. Distinctive colonial types of morphological mutants have been described as "button" (*11*), "colonial" (*1*), or "cauliflower" (*8, 13*), and several have been shown to differ from the wild type by single genes (*1, 11*).

It has been found that on the addition of certain chemicals to culture media the morphology of wild-type cultures of *Neurospora* will change so that they closely resemble in growth habit certain genetically determined colonial types. These phenomena are obviously important because of their possible relation to the mechanism of gene-determined morphogenesis in fungi, and also because of their practical utility in handling these microorganisms. The term *paramorph* is proposed to designate a fungus culture in which morphological changes have been environmentally induced without any corresponding genetic alterations. It is thought that paramorph (paramorphic phenocopy) or *paramorphic culture* more exactly expresses the desired meaning than the term *phenocopy* as used by Goldschmidt (*7*). Phenocopy has been applied to environmental duplication of specific gene effects on development. Paramorph refers to a *class* of morphological changes in a less differentiated organism. Since *paramorphogenic* activity would involve an environmentally induced qualitative change in growth habit, it is to be distinguished from toxicity effects in which a colony may grow to a limited extent before it is killed by a chemical. In the experiments here described, the colonial paramorphic cultures transplanted to inhibitor-free medium have always grown, and have resumed their normal growth habit.

Littmann (*12*) observed that the addition of 1.5 percent dehydrated ox gall to culture media restricted mycelial growth of certain fungi. Since desoxycholic acid is a normal constituent of ox bile, we have tested the effects of the addition of sodium desoxycholate to culture media, and have found that it restricts the growth of *Neurospora* and the mucoraceous fungus *Syncephalastrum racemosum* to a compact thallus or colony. Further tests have shown that a number of other agents added to either liquid or solid media are likewise capable of producing colonial paramorphs in these fungi. Typical results obtained with some of these substances are given in Table 1. The most suitable concentration of sodium desoxycholate for the production of small discrete colonies of either fungus is 0.03 to 0.035 percent (9×10^{-4} molar) in medium buffered to pH 7.0.

Since sodium desoxycholate is surface-active, another anionic surface-active agent, Tergitol 7[2] (sodium 3,9-diethyltridecanol-6 sulfate) was tested and found to induce similar growth changes in both organisms. The most suitable concentration was 0.00175 percent (5×10^{-5} molar) when added to autoclaved medium.

The observation that inositol-requiring mutant strains of *Neurospora* grown in suboptimal concentrations of inositol form pellets in liquid culture (*2*), and form restricted colonies on agar media, suggested the possible relation of inositol to the paramorphic effects described above. Since gammexane (γ-hexachlorocyclohexane) has been shown to be a specific inhibitor of certain biological and enzymatic activities of inositol (*5, 9, 10*), the colonial paramorphogenic activity of gammexane was investigated. As shown in Table 1, this compound, added to either minimal or complete agar media in a concentration of 0.025 percent, was found to be a moderately effective paramorphogenic agent for *Syncephalastrum*. Its effect on wild-type *Neurospora* is much less marked, giving only a slight modification of morphology and no appreciable change in growth rate. These effects of gammexane cannot be reversed by inositol.

L-Sorbose, the keto-hexose listed in Table 1, is also paramorphogenic, but differs from the other compounds in that much higher concentrations are required for activity. In addition, colonies on sorbose-containing agar media remain restricted in growth for

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[2] Sample obtained by courtesy of Carbide and Carbon Chemical Corporation.

periods up to 15 days, while with the other compounds the cultures tend to form aerial mycelia earlier. The paramorphogenic effect of sorbose can be reversed, apparently competitively, by other sugars; glucose, sucrose (see Fig. 1), and more slowly by maltose and by mannose. Other compounds tested, including glycerol, fructose, lactose, starch, and galactose, are much less active in reversing the sorbose effect, although they are used by *Neurospora* as carbon sources.

The paramorphogenic activity of sorbose for both *Neurospora* and *Syncephalastrum* is evidence against a direct relation of surface activity to colonial paramorphogenic activity in all cases. This view is also supported by the additional findings that paramorphogenic activity of surface-active agents is limited to certain anionic compounds. Each of the compounds listed below has a surface tension activity between 25 and 40 dynes per cm for a one-percent solution (*6*); yet their paramorphogenic activities vary both quantitatively and qualitatively. Of the anionic agents, sodium lauryl sulfate and aerosol OT (dioctyl sodium sulfosuccinate) produced colonial paramorphs at concentrations comparable to effective concentrations of Tergitol 7 and sodium desoxycholate, whereas sodium oleate was inactive. The cationic surface-active agents tested, Roccal (alkyl-dimethyl-benzyl-ammonium chloride) and Isothan 15 (lauryl isoquinolinium bromide), were very toxic, but showed no paramorphogenic activity. Tween 80 (polyoxyethylene derivative of sorbitan monooleate), the only nonionic surface active agent tested, was not toxic at concentrations up to

TABLE 1

EFFECTS OF PARAMORPHOGENS ON *Neurospora* AND *Syncephalastrum* CULTURES

Compound	Concentration	Medium			96-hr growth on solid medium*	
		Type	pH	Carbon source	*Neurospora*†	*Syncephalastrum*‡
	%			%		
Sodium desoxycholate	.01	min.	7.0	1.0 S	spreading	
	.02	min.	7.0	1.0 S	SR	R—5 mm
	.03	min.	7.0	1.0 S	R	
	.03	compl.	7.0	1.0 S	R—30 mm	
	.03	compl.	7.0	0.1 S		R—5 mm
Tergitol 7	.001	min.	5.5	1.0 S	R—10.0 mm	
	.0015	min.	5.5	1.0 S	R— 2.5 mm	
	.002	min.	5.5	1.0 S	R— 1.0 mm	
	.002	min.	5.5	0.1 S	R	R—2 mm
	.002	compl.	5.5	0.1 S	SR	spreading
	.004	MDA	4.7	4.0 D		R—4 mm
	.04	MDA	4.7	4.0 D		R—1 mm
Gammexane	.025	min.	5.5	0.1 S	SR	R—1 mm
	.025	compl.	5.5	0.1 S	SR	R—4 mm
	.05	min.	5.5	0.1 S	SR	
	.05	MDA	4.7	4.0 D		R—2 mm
L-Sorbose	1.0	min.	5.5	0.1 S	R	R—2 mm
	1.0	compl.	5.5	0.1 S	R	R—2 mm

* Characterization of growth, or average colony diameter in mm.

† Normal growth rate: 4.0 mm per hr. Similar results obtained with 5 lines tested; *N. crassa* SY4a, Abbott 12A, Chilton a, *N. tetrasperma*, and *N. sitophila* 299-pyridoxinless (pyridoxin added to minimal medium).

‡ Normal growth rate: 0.7 mm per hr. Average of 4 strains; 479 New Britain S. W. P., 527 Quebec, Canada, 551 Columbia University Culture Collection, and 742 Panama Canal Zone.

R—restricted growth
SR—slightly restricted growth
S—sucrose
D—Dextrose
MDA—minimal dextrose asparagine agar
min.—Neurospora minimal agar
compl.—Neurospora minimal agar supplemented with vitamins, enzyme-hydrolyzed casein, and yeast extract

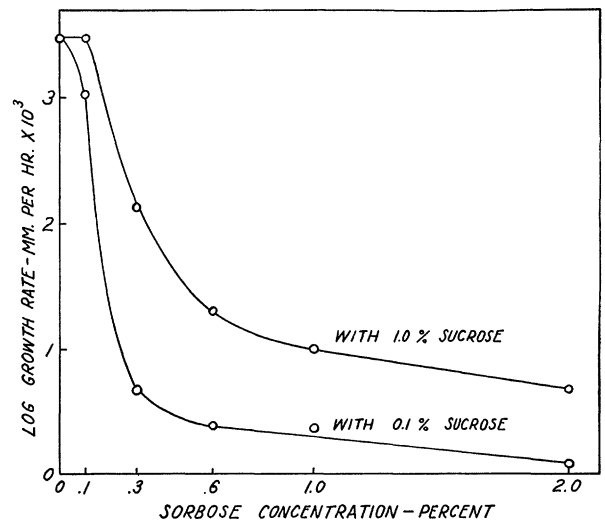

FIG. 1. Effect of sucrose on the paramorphogenic activity of sorbose on *N. crassa*. Linear growth rates determined at 25° C in growth tubes. Note differences in growth rate on two levels of sucrose.

one percent, and was inactive as a paramorphogen. There is no evidence that inositol is directly involved in the paramorphogenic activities of the other chemicals, since high concentrations of inositol have no effect on paramorphogenesis by sodium desoxycholate, Tergitol, or sorbose with *Neurospora,* or on that by desoxycholate with *Syncephalastrum.* Furthermore, bio-assays of *Neurospora* mycelium grown in the presence of Tergitol 7, or of sorbose, showed no significant variations from the normal inositol content. In addition, sorbose has no significant effect on the requirement of the *inositolless* mutant for inositol. These findings suggest that the paramorphogenic agents tested do not influence either inositol utilization or inositol synthesis by *Neurospora* or *Syncephalastrum.*

In addition to theoretical considerations, the use of paramorphogens provides a new and valuable tool for handling many colonies on microbiological plating media. Sorbose has been selected as the standard colonial paramorphogen, because colonies on sorbose show no tendency to form extensive aerial mycelia, and because, in comparison with the other compounds, the effective concentration range of sorbose is wide, and survival is significantly better. Media containing 0.8 percent sorbose and 0.1 percent sucrose or 0.2 percent sorbose and 1.0 percent glycerol have been used effectively. The value of the sorbose technique in mutation studies has been demonstrated, using a strain of *Neurospora* which has a wild-type growth rate and which produces exclusively uninucleate microconidia (*1*). Microconidia were treated with X-rays (20,000 r) and plated directly into complete medium containing sorbose. Nineteen hundred colonies arising from the treated microconidia were isolated and examined for mutants; 24.2 percent morphological variants and 3.1 percent biochemical mutants were recovered, with considerably less labor than is required by other techniques. In 500 control isolates 0.2 percent morphological and no biochemical mutants were recovered.

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Purine Metabolism in *Tetrahymena* and Its Relation to Malignant Cells in Mice[1]

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ONE OF THE PRIME GOALS of studies involving metabolic inhibitors has been the discovery of useful chemotherapeutic agents which could be directed against invading organisms of man. Accordingly, if tests on bacteria *in vitro* seemed promising the compound was subjected to tests on the same microorganisms and others *in vivo.* When metabolic inhibitors which blocked reactions peculiar to the invader but not to the host were discovered (e.g. sulfonamides for *p*-aminobenzoic acid), then useful treatment resulted. Following these initial findings (*6, 18*), a long list of compounds have been tailored to inhibit many natural metabolites, and great strides have been made toward an understanding of competitive inhibition of specific enzymes by antimetabolites (*19*).

While certain encouraging reports have appeared in the literature concerning the retardation of the growth of neoplastic tissue following the injection of folic acid antagonists (*5, 15, 17*), nitrogen mustards

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