Colonial Growth of Neurospora

Sorbose and enzymes alter the composition of the cell wall and induce morphological changes.

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Abstract. L-Sorbose, an agent which induces colonial growth in *Neurospora crassa,* also induces structural changes in the cell wall. Acid hydrolyzates of cell walls isolated from sorbose-grown (colonial) hyphae contain more glucosamine and less glucose than do hydrolyzates of cell walls obtained from normally growing hyphae. Snail digestive juice, an agent which effects a structural change in the cell wall of *N. crassa* by liberating from it large quantities of glucose, has been found to induce colonial growth.

In 1949 Tatum *et al.* (*1*) described the induction of colonial growth in *Neurospora* by addition of L-sorbose or certain surface-active agents to the culture medium. The vegetative hyphae of *N. crassa* normally exhibit the growth pattern shown in Fig. 1*a*, in which branching occurs at intervals behind the hyphal tip. The hyphae are bounded by a cell wall and divided by incomplete crosswalls into cylindrical, multinucleate cells. In the presence of L-sorbose or other of the so-called "paramorphic agents," the mycelium grows in dense colonies consisting of very short cells (Fig. 1*c*). The amount of branching is greatly increased, with branches frequently occurring at the hyphal tip itself, a phenomenon infrequently seen in normally growing cultures. Many colonial mutants of *N. crassa* have been described (*2*).

The cell wall must obviously play a crucial part in determining the morphology of *Neurospora,* for spherical, osmotically sensitive protoplasts may be formed from *Neurospora* hyphae by treatment with snail digestive juice (*3*), a mixture of enzymes in which carbohydrases predominate (*4*). Work on bacteria has shown that characteristic aberrations in morphology can occur in the presence of agents thought to interfere with cell wall metabolism (*5*). One might suspect that specific deviations from normal cell wall structure in *Neurospora* could give rise to equally specific deviations in morphology and growth pattern, such as those found in colonially growing hyphae. In order to investigate this possibility, the cell walls of normal and colonial (sorbose-grown) hyphae were compared with respect to carbohydrates released by acid hydrolysis; using the opposite experimental approach, we attempted to induce characteristic changes in morphology and growth pattern by exposure of normally growing hyphae to an enzyme preparation capable of producing a structural change in the cell wall.

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Analysis of Reducing Sugars

Young, healthy mycelia of wild-type strain SYR 9-7a were grown in liquid culture on 0.2 percent sucrose-minimal medium (*6*), with and without addition of 4 percent sorbose. The mycelial pads were rinsed, lyophilized, pulverized in a mortar, and extracted for 15 to 20 hours with 1-percent deoxycholate. This treatment yielded cell wall preparations which were almost always free of cytoplasm, as judged by microscopic examination. The occasional contaminated preparations were discarded. The walls were then rinsed several times with distilled water, passed up through a graded alcohol series, and dried out of absolute ethanol. Ten-milligram samples were hydrolyzed for 30 min in a water bath at 100°C in 1 ml of 3*N* HCl. After removal of the residue by centrifugation, the hydrolyzate was depleted of excess HCl by flash evaporation and made up to 2 ml. One-tenth-milliliter aliquots were analyzed by paper chromatography, by use of an *n*-butanol–propionic acid–H_2O solvent; reducing sugars were detected with a *p*-anisidine spray (*7*). Chromatograms of wall hydrolyzates from normal and colonial (sorbose-grown) hyphae, prepared according to the methods described above, revealed the presence of only two sugars, glucose and glucosamine. These findings are similar to those obtained by other workers who have examined the cell wall carbohydrates of *Aspergillus* (*8*) and *Allomyces* (*9, 9a*).

The glucosamine spot suggested the presence of chitin; this was confirmed by use of a modification of the Van Wisselingh test for chitin (*10*), which was applied to wall material isolated from normal and colonial (sorbose-grown) hyphae.

The presence of a polymer containing only glucose was revealed by incubation of isolated cell walls of normal hyphae with snail digestive juice (Industries Biologiques Françaises). Paper chromatography of soluble sugars in such digests revealed that large quantities of glucose had been liberated from the isolated walls. All other reducing sugars were absent, including N-acetylglucosamine. Tests in which purified chitin was incubated with snail digestive juice and the supernatant solution examined by paper chromatography showed no release of N-acetylglucosamine, and thus suggested that the particular preparation of snail digestive juice used in these experiments did not contain an active chitinase.

These results suggest that at least two carbohydrate polymers are present in the cell wall of *N. crassa.* The existence of more than two polymers cannot be excluded by the information obtained so far.

Aliquots of acid hydrolyzates prepared from isolated cell walls of normal and colonial (sorbose-grown) hyphae were quantitatively analyzed for glucose and glucosamine. Glucose was determined by a colorimetric test utilizing diphenylamine (*11*, p. 78) and glucosamine by the method of Dische and Borenfreund (*11*, p. 98). The results presented in Table 1 show a chemical difference between the cell walls of normal and sorbose-grown hyphae. With respect to sugars liberated by a 30-min acid hydrolysis, the walls of colonial (sorbose-grown) hyphae contain 184 percent as much glucosamine and 68 percent as much glucose as do the walls of normally growing hyphae. The ratio of glucosamine/glucose was 0.12 for normal hyphae and 0.32 for sorbose-grown hyphae. The total amount of carbohydrate released by acid hydrolysis from the walls of sorbose-grown hyphae was ap-

proximately 80 percent of that released from the walls of normally growing hyphae. It is not yet known whether this difference is caused by variations in the quantity of some unknown wall component or by differences in the hydrolysis rates of the polymers which comprise the cell wall.

These results are of special interest in view of the well-known observation that the aerial hyphae of sorbose-grown colonies possess normal morphology, for this fact suggests that sorbose acts at the cell surface. The cytoplasm of the aerial hyphae is continuous with the cytoplasm of the colonial hyphae lying in contact with the agar, and the aerial hyphae cannot grow except by utilizing nutrients transported from these colonial hyphae. One might therefore think that if sorbose were acting inside the cells the morphological effect would be transmitted to the growing aerial hyphae through the colonial hyphae with which they are continuous. Another indication that sorbose may act at the cell surface comes from the preliminary experiments of Wilson (*12*), who observed that the growth of hyphae injected with sorbose was completely normal. Calculations showed the amount of sorbose injected to be greater than that normally found (by studies with C^{14}-sorbose) in hyphae during sorbose-induced colonial growth.

The induction of colonial growth by sorbose does not appear to involve a large-scale uptake or incorporation of this compound into the mycelium, for pre-formed mycelial pads (dry weight 5 to 6 mg) induced to grow colonially by transfer to a sucrose-sorbose-C^{14} medium contained an amount of radioactivity which indicated that approximately 1 μg of sorbose was present in the mycelium after 8 hours of growth in the presence of the labeled compound.

Induction of Colonial Growth by Snail Digestive Juice

Bachman and Bonner (*3*) have described the production of protoplasts in liquid cultures of wild-type *N. crassa* by snail digestive juice at a concentration of 10 percent. Their results show that snail digestive juice is presumably capable of weakening the *Neurospora* cell wall in vivo. The large-scale liberation of glucose from isolated walls by snail digestive juice has been described in the first section of this report. The induction of morphological changes in *Neurospora* by this agent is described in the following paragraphs.

Table 1. Sugars released by acid hydrolysis from 10 mg of isolated cell walls of strain SYR 9-7a. Values corrected for addition of water and removal of N-acetyl group during hydrolysis.

Sugars	Medium: 0.2% sucrose			Medium: 0.2% sucrose and 4.0% sorbose		
	Wall prep. 1	Wall prep. 2	Mean, 1 + 2	Wall prep. 1	Wall prep. 2	Mean, 1 + 2
Glucosamine (mg)	0.50	0.64	0.57	1.00	1.09	1.05
Glucose (mg)	4.90	4.61	4.76	3.06	3.46	3.26
Glucosamine + glucose (mg)	5.40	5.25	5.33	4.06	4.55	4.30
Ratio: glucosamine/glucose	0.10	0.14	0.12	0.33	0.32	0.32

Agar plates of 2-percent sucrose-minimal medium were made up with concentrations of snail digestive juice that increased by steps of 1 percent from 1 through 5 percent. To minimize denaturation, the digestive juice was not added until the agar had cooled to 55°C. Streptomycin and penicillin, which by themselves have no effect on morphology, served to prevent bacterial growth. Plates were then inoculated with a suspension of conidia from strain SYR 9-7a.

With increasing concentrations of snail digestive juice, there was a progressive decrease in colony diameter and a

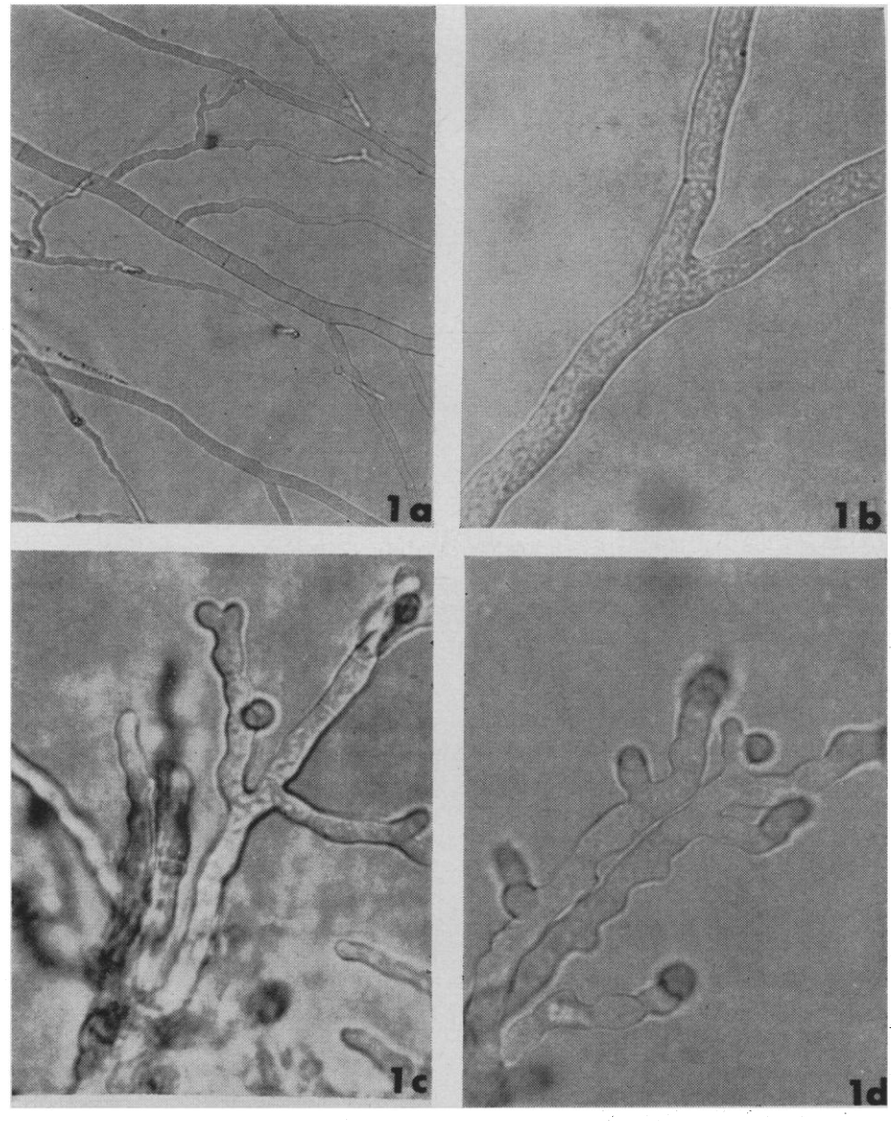

Fig. 1. *a*, Hyphae of wild-type strain SYR 9-7a growing on 2 percent sucrose-minimal agar (× 125). *b*, Same as *a* (× 500). *c*, Hyphae of wild-type strain SYR 9-7a growing on sorbose agar; 1.5 percent sorbose, 0.2 percent dextrose, 0.2 percent glycerol, minimal medium (× 500). *d*, Hyphae of wild-type strain SYR 9-7a growing on 2 percent sucrose-minimal agar containing 3 percent snail digestive juice (× 500).

corresponding increase in extent of branching until at a concentration of 5 percent (and to a somewhat lesser extent at 3 percent and 4 percent) the cultures were macroscopically indistinguishable from those grown in the presence of high concentrations of sorbose. Microscopically, the hyphae strongly resembled sorbose-grown hyphae (Figs. 1*c* and 1*d*), although marked variations in morphology and extent of branching were sometimes found in different parts of the same colony. The ability of snail digestive juice to induce colonial morphology declined with increasing age of the enzyme preparation. At a concentration of 10 percent snail digestive juice, growth did not occur. It is of special interest that all the typical features of colonial morphology as observed in sorbose-grown cultures can be induced by snail digestive juice, including initiation of branches at the hyphal tip, increase in branching, and some shortening of cell length. Growth was normal in the presence of autoclaved snail digestive juice, as well as serum albumin, N-Z-Case, and various proteolytic enzymes. Not enough is known about the mechanism by which branches are produced to explain these results, especially in view of the complex enzymatic content of snail digestive juice. In the absence of much sorely needed information, one can only speculate that a weakening of the cell wall by enzymatic digestion, in association with the high internal pressure known to exist in *Neurospora* hyphae (*12*), could lead to an increase in the number of branches initiated. The results described above suggest that sorbose may induce colonial growth in a similar manner, perhaps by inhibiting synthesis of the glucose polymer in the cell wall.

The results reported herein show that, in some cases at least, colonial morphology occurs in association with changes in cell wall structure. However, these observations cannot be placed in their proper perspective until further information is available on normal wall structure, cell wall metabolism in the presence of sorbose, and wall structure of some other colonial forms of *Neurospora,* notably the colonial mutants (*13*).

References and Notes

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13. This work was performed during the tenure by N. de Terra of a U.S. Public Health Service postdoctoral fellowship of the National Cancer Institute.

21 June 1961

Measurement of the Relations between Chromosomes and Behavior

Abstract. The relations between the three major *Drosophila melanogaster* chromosomes and individual differences in geotaxis are assayed in populations selected for positive and negative geotaxis and in an unselected foundation population. The major changes which occur with selection and the differential roles in geotaxis of the three large chromosomes are described.

Experimental behavior genetics has usually been limited to selective-breeding and strain-comparison studies (*1*). In a few experiments the effects of known loci on behavior have been analyzed (*2*). Recently it has been found that it is feasible to study the relations between variations in sets of chromosomes and individual differences in behavior (*3*). Efficient, reliable, and objective methods developed for the observation of behavior in populations can now be combined in behavior genetic analysis with genetic techniques developed for assaying chromosomes. Such analysis can yield information about both the relative importance of particular chromosomes in producing individual differences in behavior and the nature of the relations between each chromosome and a behavior.

In the study reported here (*4*) the role in geotaxis of the three major *Drosophila melanogaster* chromosomes and their several interactions has been assayed with a multiple inversion tester stock (*5*) in a 2^8 factorial breeding experiment, reported in detail elsewhere (*6*). The chromosome assay method described by Mather and Harrison (*7*) depends on comparison of the geotactic effect of the chromosomes from a population under study with that of their homologues from a common tester stock. The homologous chromosomes of different populations under study can then be compared with one another by means of this common standard of reference. Direct comparison of the chromosomes from different populations is impossible because the chromosomes cannot ordinarily be identified and followed in segregation. The chromosomes of the tester stock contain inversions, however, which reduce recombination in heterozygotes; they also contain dominant morphological marker genes, which make it possible to follow in a backcross the segregation of these chromosomes from their homologues.

The assay breeding procedure consists of crossing a multiple inversion tester stock to the population being tested and then backcrossing the resulting F_1 hybrid to the same population. It produces individuals having either of two chromosome combinations for each of the three major *D. melanogaster* chromosome pairs. A pair of chromosomes is thus structurally either heterozygous or homozygous—that is, either the inversion chromosome from the standard tester stock is paired with its homologue from the tested population or both homologues come from the tested population. From the difference in geotactic behavior between the structural heterozygotes and the structural homozygotes an estimate is obtained of the geotactic effect of a given chromosome from a tested population.

The observations in this study are distributions of geotactic scores in the mass screening maze (*8*) for the eight combinations of structural heterozygotes and structural homozygotes produced by each assay. The maze affords objective, automatic, and reliable mass screening measurements of individual differences in both positive and negative geotaxis in populations under constant stimulus conditions.

Selective-breeding analysis (*6, 8*) has previously shown that the variance in individual differences in geotaxis contains a large genetic component and that the sign of this taxis is a property of the individual genotype. Geopositive and geonegative populations have been developed by selection from a heterogenic foundation population (*6, 8*) which has itself remained geotactically polymorphic while being maintained as