ADAPTATION IN PANTOTHENATE-REQUIRING NEUROSPORA
II. NUCLEAR COMPETITION DURING ADAPTATION[1]

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ABSTRACT

DAVIS, R. H. (U. Michigan, Ann Arbor.) *Adaptation in pantothenate-requiring Neurospora. II. Nuclear competition during adaptation.* Amer. Jour. Bot. 47(8): 648–654. Illus. 1960.—The process of adaptation in pantothenate-requiring *Neurospora* was studied by the use of heterocaryons constituted of the *pan-1* strain and a modified strain, *pan-1 m*, derived from it. Although *pan-1-m* homocaryons grow well on a pantothenate concentration on which *pan-1* grows little or not at all, *pan-1* nuclei often have a selective advantage in *pan-1* + *pan-1-m* heterocaryons grown on the same medium. This results in non-adaptive changes in nuclear ratios and labile growth rates of the heterocaryons. Nuclear competition does not occur when pantothenate is not limiting to the growth of either homocaryon. The results are discussed, and related to past work on adaptation and nuclear ratio changes in *Neurospora* hetercaryons.

ADAPTATION in *Neurospora*, in the sense of genetic changes in the nuclear population of a mycelium which lead to more rapid growth, has been by no means thoroughly investigated. The coenocytic organization of *Neurospora* mycelia allows numerous nuclei to cooperate in the activities of a single cytoplasmic unit. Through mutation or through the fusion of genetically dissimilar mycelia, a heterocaryon may arise in which there is a heterogeneity in the nuclear population. Mutant nuclear types which will not sustain normal growth in pure cultures may, when combined in a "balanced heterocaryon," contribute to a normal phenotype through the mutual complementation of the wild type alleles of the mutant genes involved. Since the nuclei in heterocaryons are not autonomous, but interact with one another in an integrated cellular system, the change of nuclear frequencies is a more complex phenomenon than adaptation in populations of uninucleate, unicellular organisms such as bacteria. The change in nuclear frequencies of an adapting *Neurospora crassa* heterocaryon is the subject of this paper.

Previous investigators have brought forth 2 different proposals regarding the interactions between the 2 components of a balanced heterocaryon. Beadle and Coonradt (1944) suggested, though without definitive evidence, that nuclear ratios are altered by selection among the hyphae at the growing frontier of a mycelium. They assumed that growth rates of individual hyphae are determined by their own nuclear ratios, and that selection for those hyphae with ratios optimal for growth leads to the establishment of an optimal growth rate (and nuclear ratio) for the heterocaryon as a whole.

Pittenger and Atwood (1956), after a more extensive investigation of this matter, maintained that hyphal selection "rarely, if ever, operates" to alter the nuclear ratio of a fully established heterocaryon, even in cases in which change of nuclear ratio would result in higher growth rate. This idea is supported by experiments in which balanced heterocaryons of biochemically mutant nuclear types, carefully synthesized with highly disparate nuclear ratios, grew at stable submaximal rates for long periods of time. The submaximal growth rate was referable to the scarcity of 1 of the 2 nuclear types. The stability of nuclear ratios was presumed to depend upon equal division rates of the nuclear types, dependence of growth rates of hyphae at the mycelial frontier upon the activities of the larger nuclear population proximal to them, and continual mixing of the nuclear population through cytoplasmic streaming and hyphal anastomosis.

A phenomenon which was not, apparently, encountered in either of the above-mentioned investigations was described by Ryan and Lederberg (1946; cf. Ryan, 1946). Adaptations of the leucineless (*leu-1*) strain of *Neurospora* to limiting concentrations of leucine were observed to take place through the occurrence and proliferation of back-mutant (*leu-1*$^+$) nuclei. It was found, however, that the *leu-1* mycelium often inhibited the growth of *leu-1*$^+$ sectors in limiting concentrations of leucine, leading to non-adaptive changes in nuclear ratios (i.e., changes less favorable to high growth rate) of a *leu-1* + *leu-1*$^+$ heterocaryon. Data reminiscent of this behavior were reported by Emerson (1947, 1948) and by Emerson and Cushing (1946), working on adaptations to sulfonamides. Such behavior cannot be accounted for in terms of simple hyphal selection or stability of nuclear ratios.

Another case of non-adaptive changes in the nuclear ratio of a heterocaryon is described in this paper. The heterocaryon is constituted of a normal pantothenic-less strain, *pan-1*, and a modified strain, *pan-1 m*, described previously (Davis, 1960). The 2 strains, isolated asexually from a single panto-

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thenic-less mycelium which had adapted to limiting pantothenate, differ by 1 known gene. The modified strain, like the normal strain, probably synthesizes little or no pantothenate endogenously, but is able to utilize pantothenate at lower concentrations than the normal strain. The latter characteristic allows mycelial sectors of *pan-1 m* to develop in cultures of *pan-1* which have ceased growth. An investigation of growth rates and nuclear proportions of heterocaryons constituted of the 2 nuclear types is reported and applied to an understanding of the process of adaptation in *Neurospora* heterocaryons.

MATERIALS AND METHODS.—The strains of *N. crassa* used were *pan-1, al-1, A* (5531, 4637; pantothenic-less, albino), a spontaneous mutant, *pan-1, al-1, m, A*, derived from it by asexual isolation (Davis, 1960), and *nic-2, al-2, A* (4540, 15300; nicotinic-less, albino). The 2 pantothenic-less strains are referred to throughout as *pan* and *pan-m*.

Fries *Neurospora* medium (Ryan, 1950) with 1.0% sucrose was used for all growth tests. This medium was supplemented with either of 2 concentrations of calcium pantothenate: (a) with 2.0 μg. pantothenate/ml., it is referred to as unlimiting pan medium; (b) with 0.2 μg. pantothenate/ml., it is referred to as limiting pan medium. The medium used for plating conidia was the synthetic crossing medium of Westergaard and Mitchell (1947), modified to contain 0.1% sucrose and 1.0% sorbose, and supplemented with 2.0 μg. pantothenate/ml. In the case of solidified media, agar was added in a concentration of 2.0%.

Dry weights of mycelia were determined after harvesting cultures grown in 10 ml. or 25 ml. medium, squeezing the mycelia between filter papers, and drying them for 18–24 hr. at 60°C. before weighing. Growth rates were measured by the tube method of Ryan et al. (1943). For all growth tube experiments, heterocaryons of *pan* and *pan-m* were synthesized according to the method of Pittenger et al. (1955). This method involves mixing 2 homocaryotic conidial types, centrifuging them to a dense pellet and allowing the pellet to grow on supplemented medium for a day before using it to inoculate a growth tube. In experiments involving Petri-dish cultures, heterocaryons were derived from single heterocaryotic condia or from sectors of the growing frontiers of heterocaryotic mycelia.

Nuclear frequencies were derived from the frequencies of conidial types isolated from heterocaryotic mycelia. In the case of heterocaryons grown on limiting pan medium, in which conidia were not numerous, samples of heterocaryotic mycelia were allowed to conidiate for 24–48 hr. in small culture tubes containing solidified unlimiting pan medium, and the conidia developed were used for frequency determinations. Conidia were collected in sterile water, filtered through glass wool, and spread on plating medium. After incubation for 2–3 days, 40–90 conidial colonies were removed randomly and transferred, 4 to a plate, to the edge of Petri dishes containing limiting pan medium. The colonies were scored with regard to the amount of growth taking place after 24 hr. Unlike *pan-m* and heterocaryotic colonies, *pan* colonies fail to grow significantly during or after this interval. Colonies were classed only with respect to the frequency of *pan* colonies, since *pan-m* and heterocaryotic colonies were not distinguishable in every case. The frequency determinations were often done in duplicate, and were found to be reproducible within 10% in all but a few cases.

In order to estimate the frequency of *pan* nuclei from the frequency of *pan* conidia, the random distribution hypothesis of Prout et al. (1953) was applied. By counts of stained conidia (Huebschman, 1952), the frequencies of conidia containing 1, 2, 3, . . ., etc. nuclei of several heterocaryons and of the 2 homocaryons was obtained. Assuming random distribution of the 2 nuclear types in the conidia, the frequency of the *pan* conidial type was plotted as a function of the frequency of *pan* nuclei. A single curve was used for all derivations of *pan* nuclear frequency, since the nuclear number distribution was found to be uneffected by nuclear ratio. The proportion of heterocaryotic conidia isolated from all heterocaryons indicated that any deviation from random distribution was not extreme. However, as homocaryotic conidia are occasionally produced in excess of expectation (Atwood and Mukai, 1955; Klein, 1958), the nuclear frequencies given must be thought of as maximal estimates of *pan* nuclear frequency and will be denoted EMP.

RESULTS.—*Response of the heterocaryon to various pantothenate concentrations.*—The conidia from a single *pan* + *pan-m* heterocaryon were used to

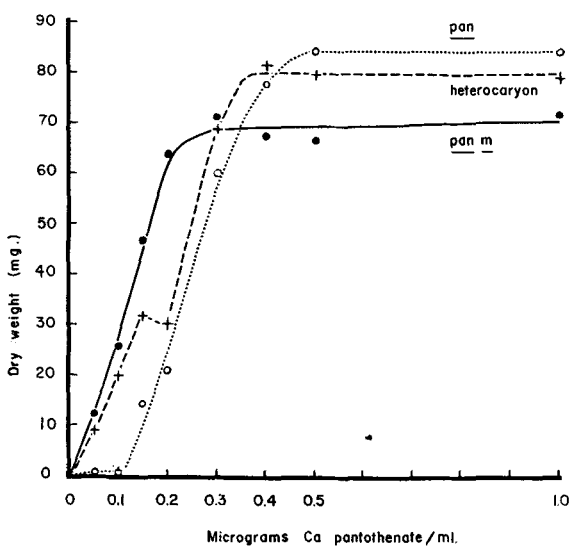


Fig. 1. Dry wt. of the homocaryons, *pan* and *pan-m*, and of the *pan* + *pan-m* heterocaryon after 6 days growth in various concentrations of calcium pantothenate. Each point represents the average of 2 cultures grown in 25 ml. medium.

inoculate media of various pantothenate concentrations, and the dry wts. developed after 6 days were compared to those of similar cultures of the 2 homocaryotic strains. The results (fig. 1) show that while the responses of the homocaryons are almost linear in their submaximal ranges, that of the heterocaryon is not. At the most limiting pantothenate concentrations, the response of the heterocaryon is similar to that of *pan-m*; at higher concentrations of pantothenate, the response changes abruptly to resemble that of *pan*. This finding indicates a dominance or selective advantage of the *pan* nuclear component at higher concentrations of pantothenate.

In a separate experiment, the EMP values of a heterocaryon grown in liquid medium containing 0.10, 0.15, 0.20, and 0.25 μg. pantothenate/ml. for 4 days were found to be 0.52, 0.50, 0.72, and 0.78, respectively, and a very similar discontinuity in dry-wt. response was again apparent. Thus *pan* nuclei are lower in frequency in heterocaryons grown in low concentrations of pantothenate than in those grown in higher concentrations. These findings must be investigated further, especially with regard to changes in EMP with time on all pantothenate concentrations. Such experiments were deferred in favor of a more detailed analysis of adaptation on a single limiting concentration of pantothenate.

Adaptation in liquid cultures.—The following experiments were designed to determine the changes of dry wt. and EMP that occurred with time in limiting pan medium. Seven series of 30 flasks each were prepared, each flask containing 10 ml. limiting pan medium. The 7 series were inoculated with the following 7 types of inocula: (1) *pan* conidia; (2) *pan-m* conidia; (3) conidia of a heterocaryon with an EMP value of 0.57; (4) *pan* and *pan-m* conidia mixed in 1:1 proportions; (5) *pan* mycelium; (6) *pan-m* mycelium; and (7) mycelium of a heterocaryon having an EMP value of approximately 0.45. The conidial inocula all contained between 1 and 2×10^4 conidia. Mycelial inocula were obtained by cutting 1–mm.^{2} blocks from Petri-dish cultures. After 3, 6, and 12 days incubation, 10 cultures of each series were harvested and weighed.

The results of this experiment (fig. 2) show that while homocaryotic cultures achieved maximal dry wt. by the sixth day of incubation, significant increments in dry wt. after the sixth day were observed in cultures inoculated with both nuclear types. In no case did the standard deviation of the mean

TABLE 1. *Dry wt. and EMP values (maximum estimates of pan nuclear frequency) of heterocaryotic cultures after various intervals of time in 10-ml. limiting pan medium, starting with different inocula*

Inoculum	Days growth	Mg. dry wt.	EMP
Heterocaryotic mycelium	0	0	0.57
	3	17.9	0.60
	6	24.7	0.25
	12	30.6	<0.10
Mixed conidia:			
1 *pan*: 9 *pan-m*	0	0	0.10
	3	21.1	0.68
	6	25.5	0.54
	12	28.3	0.56
1 *pan*: 1 *pan-m*	0	0	0.50
	3	15.1	0.79
	6	22.2	0.26
	12	29.3	0.26
9 *pan*: 1 *pan-m*	0	0	0.90
	3	12.0	0.57
	6	25.5	0.26
	12	30.2	0.15

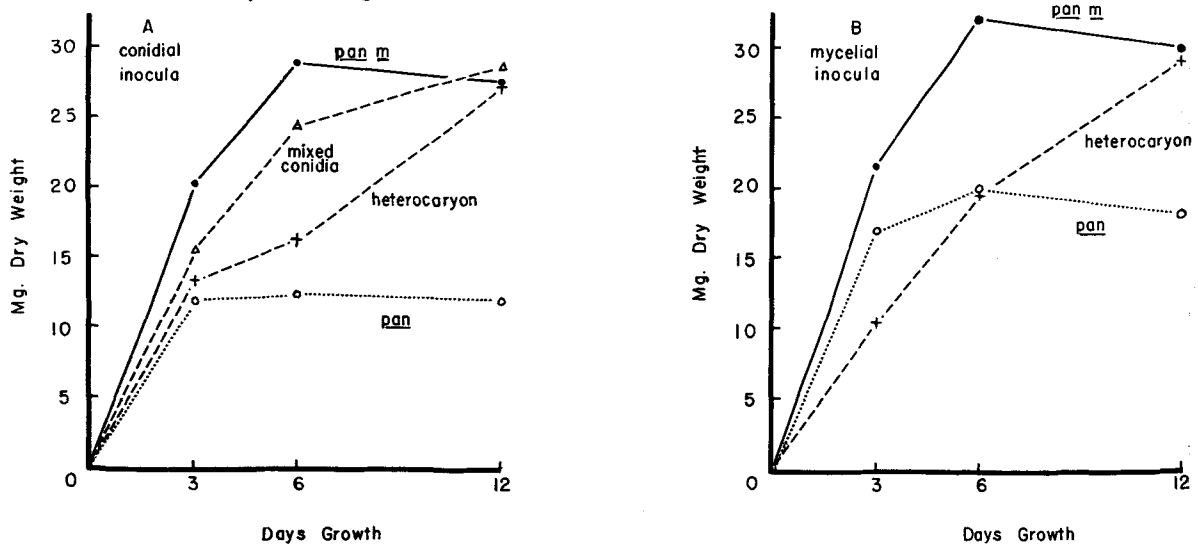


Fig. 2. Dry wt. of *pan*, *pan-m*, and mixed cultures after 3, 6, and 12 days growth in limiting pan medium. a: conidial inocula; b: mycelial inocula.

dry wt. of any series at any time exceed 1.5 mg.

A similar experiment was performed on a smaller scale, using conidia mixed in various proportions, and mycelium of a heterocaryon. EMP values as well as dry wt. were determined after 3, 6, and 12 days incubation. The results (table 1) show in most cases that *pan* nuclei decrease significantly in frequency from the third to the twelfth day of growth in limiting pan medium; in the case of inocula containing 10% *pan* nuclei, the changes in EMP during this interval are minor.

These experiments are informative in several respects: (1) when both nuclear types are represented in an inoculum, maximal dry wt. is not attained by the sixth day, as it is in homocaryotic cultures. This is true regardless of whether conidia from a heterocaryon, heterocaryotic mycelium, or a mixture of homocaryotic conidial types are used as inocula. (2) That cultures inoculated with heterocaryotic or mixed homocaryotic conidia do not grow as fast as *pan-m* cultures cannot be ascribed merely to there being fewer *pan-m* nuclei in the former inoculum-types. Separate experiments showed that *pan-m* cultures inoculated with 1/10 the number of conidia used in the present experiment were not significantly different in dry weight after 3 and 6 days growth, and always achieved maximal dry wt. by the sixth day. (3) Data regarding the changes in frequency of *pan* nuclei during the growth of heterocaryons suggest that growth of heterocaryons takes place in 2 phases. A first phase is observed in which *pan* nuclei, if low in initial frequency, increase faster than *pan-m* nuclei. The greater the representation of *pan* nuclei in the inoculum, moreover, the less the mycelium grows in the first 3 days (table 1). A second phase then emerges, in which growth continues with a concomitant reduction in the frequency of *pan* nuclei. The first phase of growth (before 3 days incubation) may be understood to some extent by the observation that *pan* homocaryons grow as rapidly or more rapidly than *pan-m* homocaryons for the first 48 hr. in this medium. This observation may be sufficient to explain the increase of EMP values of heterocaryotic cultures during the first 3 days. From the results with heterocaryotic cultures, however, it appears that the growth of the *pan-m* component is delayed appreciably during the same period by the growth or presence of the *pan* component.

Adaptation in agar cultures.—The preceding experiments, using liquid media, reveal the general pattern of growth of mixed cultures as they "adapt" to a limiting pantothenate concentration. The question of nuclear competition is not answerable with such evidence, since the relationship of the nuclear components in the mycelium and the response of the mycelium to a changing pantothenate concentration is obscure. The problem was therefore studied by the use of agar media, in which nuclear ratios and patterns of growth could be studied in the same mycelium.

The growth rates of the homocaryons and several heterocaryons grown on unlimiting pan agar medium were determined. The growth rate of *pan* ranged from 4.0 to 3.5 mm./hr. in different experiments; that of *pan-m* was generally 2.4 mm./hr. In both cases, the progress of growth was linear with time. Heterocaryons, varying in EMP values from 0.99 to 0.12,

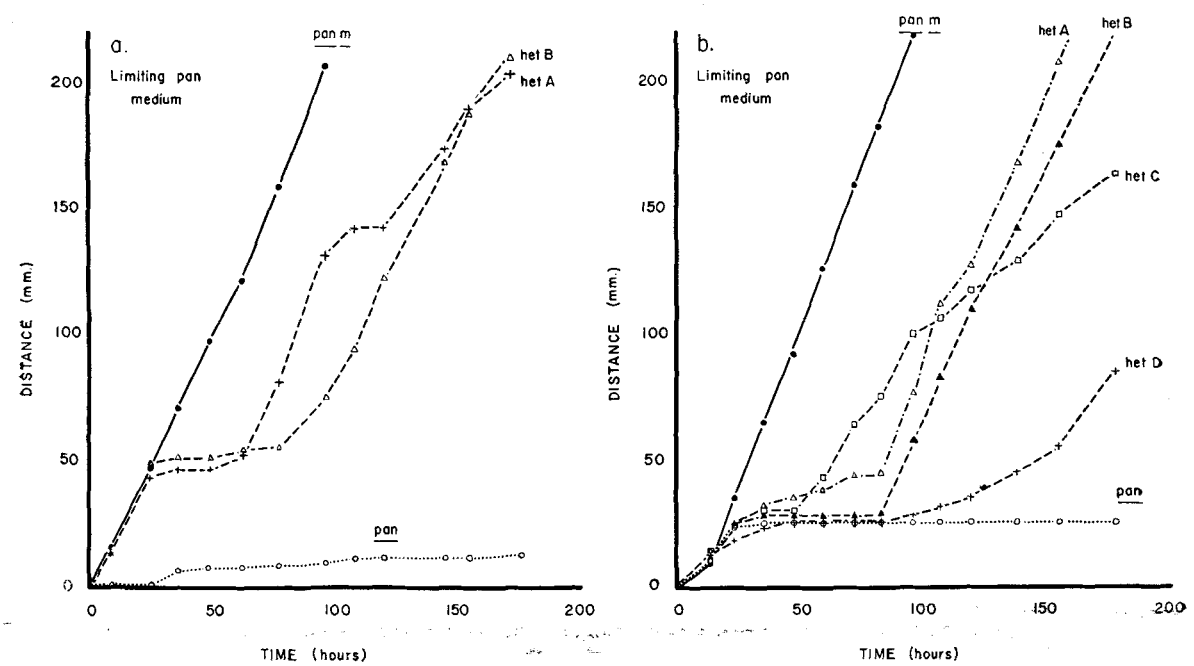


Fig. 3. Growth curves of *pan* + *pan-m* heterocaryons grown in growth tubes on limiting pan medium, together with growth curves of homocaryotic mycelia. Two separate experiments (a and b) are shown.

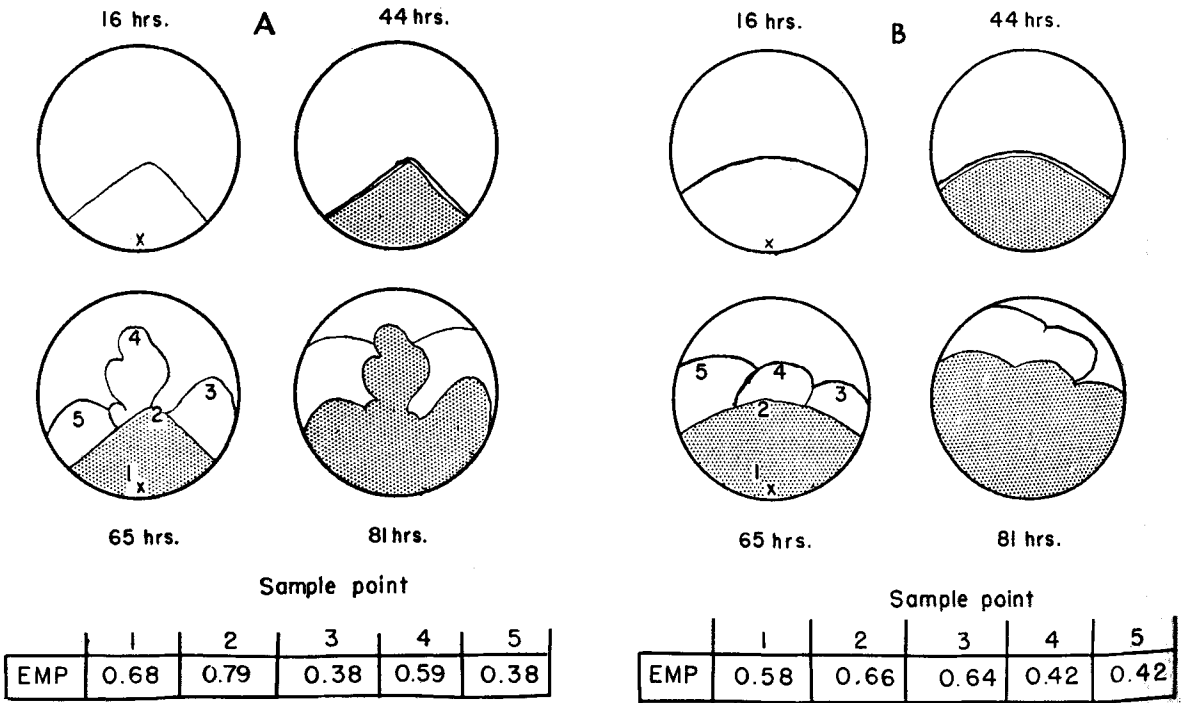
also grew linearly with time, those with lower EMP values growing at lower rates (2.4 to 2.8 mm./hr. for heterocaryons with EMP values of 0.12; 4.0 mm./hr. for those with EMP values of 0.88 and 0.99). EMP values, determined from conidial samples taken directly from 3 widely separated points by way of sampling ports along the length of the growth tube, were observed to remain constant (within 8% at least) throughout 200 mm. of growth on this medium.

The 3 types of mycelia were then grown on limiting pan agar medium (fig. 3). The growth rates of *pan-m* mycelia were approximately the same (2.3 mm/hr.) as they were when *pan-m* was grown on unlimiting pantothenate. The *pan* strain did not grow significantly, though an initial short period of growth was often observed if fresh conidial pellets were used as inocula (*pan* control, fig. 3b). The inability of the *pan* strain to grow on this medium was unexpected in view of its ability to grow on limiting pan liquid medium, but this behavior was consistently observed.

Heterocaryons grown on limiting pan agar medium in growth tubes displayed unstable growth rates. A short period of growth was always followed by a variable interval during which no growth occurred at all. This first period of growth is not very significant, since *pan* homocaryons inoculated in the same way also grew briefly at first, probably as a result of an endogenous pantothenate content. Subsequently, however, growth was renewed: (1) at a rate characteristic of *pan-m* homocaryons; (2) at a slowly increasing rate which approaches that of the *pan-m* homocaryon; (3) at a fluctuating rate intermediate between those of the homocaryons; or (4) at a high rate, followed by a second cessation of growth. In all cases, both nuclear types were present at the end of the period of growth. Growth curves showing these patterns are given in fig. 3.

It was of interest to determine whether or not changes in nuclear frequencies of heterocaryons grown on limiting pan medium could be correlated with abrupt changes in growth rates. To this end, heterocaryons were inoculated at the edges of Petri dishes containing limiting pan agar medium. A broader frontier was available for study than in a narrow growth tube, and several types of behavior could be observed in a single culture. In addition, EMP values could be determined at any desired point.

All heterocaryons tested in this manner were again observed to grow rapidly at first, and then to stop. Renewed growth of the heterocaryons arose from sectors developing along the frontier. The sectors would either continue growing or stop growing a second time, repeating the original pattern of growth. Examples of growth patterns displayed by heterocaryons grown in this way are shown in fig. 4, together with EMP values determined at different points.



Sample point

	1	2	3	4	5
EMP	0.68	0.79	0.38	0.59	0.38

Sample point

	1	2	3	4	5
EMP	0.58	0.66	0.64	0.42	0.42

Fig. 4. Two examples (a and b) of the pattern of growth displayed by *pan* + *pan-m* heterocaryons grown in 90-mm. Petri dishes on limiting pan medium. The contours of the mycelia at each observation are shown; shaded areas represent areas covered by mycelia at the preceding observation. Mycelial samples were taken after 65 hr. from the numbered points, and EMP values derived from these samples are given below the figure; "X" represents the point of inoculation.

TABLE 2. *EMP values of stationary and growing areas of heterocaryotic mycelia grown in limiting pan agar medium in Petri dishes*

Growing areas	*Stationary areas*
0.38	0.79
0.38	0.59
0.42	0.66
0.42	0.64
0.45	0.58
0.20	0.55
0.35	0.65
0.44	0.13
0.26	0.51
0.13	
0.42	
0.46	

The EMP values in different regions of the heterocaryons are correlated with the continuation or cessation of growth. In fig. 4a, the EMP values of sample points 2 and 4, in stationary areas, are considerably higher than those from points 3 and 5, in areas which continued to grow in the 16 hr. following the sampling. EMP values from stationary and growing areas (disregarding areas around inocula) of 6 heterocaryons grown in this manner are collected in table 2. EMP values in 4 of the 6 heterocaryons were determined in duplicate from adjacent mycelial samples, and the 2 determinations agreed within at least 10% in every case. With 1 exception out of 21 determinations, there is no overlapping of the ranges of EMP of growing and stationary areas. As a general rule, it is concluded that growth of a heterocaryon under these conditions is associated with EMP values less than 0.45, and that cessation of growth is associated with EMP values higher than 0.50. That the changes in EMP are not an artifact of sampling methods is indicated by the observation that EMP values determined in the same manner from heterocaryons grown in unlimiting pan medium remained constant during growth.

The agar culture experiments appear to show in the extreme the type of behavior displayed by heterocaryons grown in liquid cultures, and it is concluded that changes in nuclear frequency are generally correlated with changes in growth rate. It is probably safe to assume that the non-adaptive overgrowth of the heterocaryon by *pan* nuclei is responsible for the cessation of growth, and that the appearance and further growth of hyphae containing fewer *pan* nuclei is responsible for the development of sectors of renewed growth.

A brief test of the interactions taking place between *nic-2, al-2, A* nuclei and *pan* or *pan-m* nuclei was made with the heterocaryons *pan-m* + *nic-2, al-2* and *pan* + *nic-2, al-2*. Both types of heterocaryons had stable nuclear proportions and growth rates when grown on minimal medium, limiting and unlimiting pan media, and nicotinic acid–supplemented medium. These results suggest that the observed competition between *pan* and *pan-m* nuclei is a function of these particular nuclear types and of the concentration of pantothenate in the medium.

DISCUSSION.—Although *pan-m* homocaryons grow more rapidly than *pan* homocaryons on limiting pantothenate, the data presented show that *pan-m* nuclei are frequently selected against in *pan* + *pan-m* heterocaryons grown on the same medium. It is emphasized that the *m* locus is the site of the only known genetic difference between the 2 homocaryotic strains. It must also be remembered that changes in nuclear proportions in heterocaryons cannot be ascribed to immutable differences in division rates, since such changes did not occur during the growth of heterocaryons on unlimiting pantothenate.

The relationship of nuclear events to growth patterns of heterocaryons in limiting pantothenate must be considered carefully in attempting to understand the apparent nuclear competition. It seems reasonable to assume that pantothenate uptake into the heterocaryotic mycelium is ascribable to the activity of *pan-m* nuclei (Davis, 1960), and that the cessation of growth on limiting pan medium is connected with a scarcity of this nuclear type resulting from an overgrowth of *pan* nuclei. The non-adaptive increase of *pan* nuclei in the mycelium may take place either on a nuclear or on a cellular level: either *pan* nuclei divide at a faster rate than *pan-m* nuclei in a given cell, or cells containing more *pan* nuclei have a generally higher nuclear division rate (and growth rate) than those containing fewer.

The central question as to what governs the differential rates of increase of the 2 nuclear types in the heterocaryon before cessation of growth on limiting pantothenate remains largely unanswered. The apparent "inhibition" of *pan-m* nuclear increase by the activity of *pan* nuclei (liquid culture experiments) might indicate that *pan* nuclei have a distinct advantage in a competition for intracellular pantothenate or its derivatives (e.g., Coenzyme A), even though *pan-m* nuclei are responsible for the uptake of pantothenate from the medium. This hypothesis is supported by the observations that: (1) *pan* homocaryons grow more rapidly than *pan-m* homocaryons on unlimiting pantothenate; (2) nuclear competition does not occur in heterocaryons grown on unlimiting pantothenate; and (3) *nic-2, al-2* nuclei (carrying the pan^+ allele) fail to compete with *pan* or *pan-m* nuclei in heterocaryons. A less plausible interpretation of the data would suggest that cytoplasmic regions containing more *pan-m* nuclei may be inhibited in their growth to a greater extent than regions containing fewer by influences which normally regulate the even progress of hyphae at the frontier of a growing mycelium (Ryan et al., 1943).

After growth of a heterocaryon ceases on limiting pan medium, pronounced sectoring takes place, leading to the formation of a mycelium in which *pan* nuclei are reduced in frequency. The observation that sectors are often initiated by a small num-

ber of hyphae which grow forward from the stationary frontier indicates that selection for hyphae with nuclear proportions favorable for growth takes place (Beadle and Coonradt, 1944). The time required for such hyphae to escape the frontier may be rather long, suggesting again an inhibitory influence of the surrounding mycelium.

The results presented here show striking similarities to those obtained by Ryan and Lederberg (1946; cf. Ryan, 1946) in the case of adaptations of *leu-1* mycelia to limiting leucine concentrations. In both cases, selection against the "adapted" nuclear type in heterocaryons was observed, leading to labile growth rates. In both cases, the evidence points to an inhibition by the original nuclear type of the increase in frequency of the other. In the case of *leu-1* + *leu-1*$^+$ heterocaryons, however, tests for changes in nuclear proportions on unlimiting leucine concentrations were not reported.

A similar process of adaptation may have been responsible for certain other data in the literature. Heterocaryons with labile growth rates were encountered frequently in the work of Emerson (Emerson, 1947, 1948; Emerson and Cushing, 1946) on adaptations to sulfonamide resistance. Their results suggest non-adaptive increases of certain nuclear types during growth of heterocaryons, but changes in nuclear ratios were not specifically studied. Labile growth rates were observed, however, both in the adaptation of the sulfonamide-requiring strain to minimal medium, and in the adaptation of the wild type strain to a medium containing sulfonamide. These examples, together with cases of adaptation in leucine-less and pantothenic-less strains, involve widely varied biochemical mutants of *Neurospora*. Nuclear competition may, therefore, be a rather common phenomenon.

Such findings are interesting with regard to the experiments of Pittenger and Atwood (1956), which demonstrated a stability of nuclear ratios in certain heterocaryons, utilizing 4 nuclear types. The mutants used were all isolated by Beadle and Tatum (1945) from the same wild type stock. Though compatible in heterocaryons, the stocks used by Pittenger and Atwood had undoubtedly become somewhat heterogeneous through outcrossing and spontaneous mutation. The difference between the behavior observed by Pittenger and Atwood and that observed here may be correlated with the origin of the nuclear types, among other things. It appears that while the more distantly related nuclear types used by Pittenger and Atwood have equal rates of increase in heterocaryons, nuclei differing at one or a few loci (as in the present case) may indulge in a competition leading to non-adaptive changes in nuclear ratio. It is of interest in this connection that some wild type nuclei which did not originate through back-mutation from *leu-1* were not selected against in heterocaryons with *leu*-1 (Ryan, 1946).

Experiments to determine the physiological nature of the competition between *pan* and *pan-m* nuclei, and others to test the effect of genetic alterations in these nuclei upon their interaction are planned.

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