A GENE THAT CAUSES NATURAL DEATH IN NEUROSPORA CRASSA [1]

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NEUROSPORA genetics has contributed much to our understanding of genic action and biosynthesis through the study of mutants that are deficient in their ability to synthesize vitamins, amino acids and other metabolites. These mutants are reparable in the sense that they can be cultured on media supplemented with the required growth factors or metabolites. There are, however, mutants that will not grow or that will grow only slowly at one temperature or another even on complete medium (HOROWITZ 1950). They may be referred to as irreparable mutants (HOROWITZ 1948).

These mutants are of special interest for the following reasons. Firstly, they might be mutants involving multiple-functioned genes (DELBRUCK's discussion following paper by BONNER 1946; HOROWITZ 1948). Secondly, as yet unidentified growth factors might be detected through the study of these mutants. Thirdly, they might be deficient or defective in enzymes not functional in synthetic processes. Most morphological mutants could be of this nature. The irreparable mutant to be described in this paper seems to belong to still another category. It is characterized on the one hand by a normal growth on minimal medium in the young condition and on the other hand by an ever-decreasing growth potential under any condition tested thus far. Besides being irreparable it is therefore of interest in another respect: the vegetative growth from a single ascospore undergoes a process of ageing and natural death. Natural death is defined arbitrarily as the progressive irreversible cessation of growth due to intrinsic causes.

MATERIALS AND METHODS

This mutant, called natural death or *nd*, was obtained after ultraviolet light irradiation of conidia of an albino strain 15300(al_2)*A*. It was selected because it stopped growing as a tiny colony on sorbose plate at 37.8°C but grew larger after the plate was transferred to 25°C.

It could be crossed with all non-*nd* strains tested. Non-*nd* vs. *nd* phenotype of isolated spores was determined by testing in growth tubes at 37.8°C or 32°C. A typical segregation is shown in figure 1. Successive subcultures

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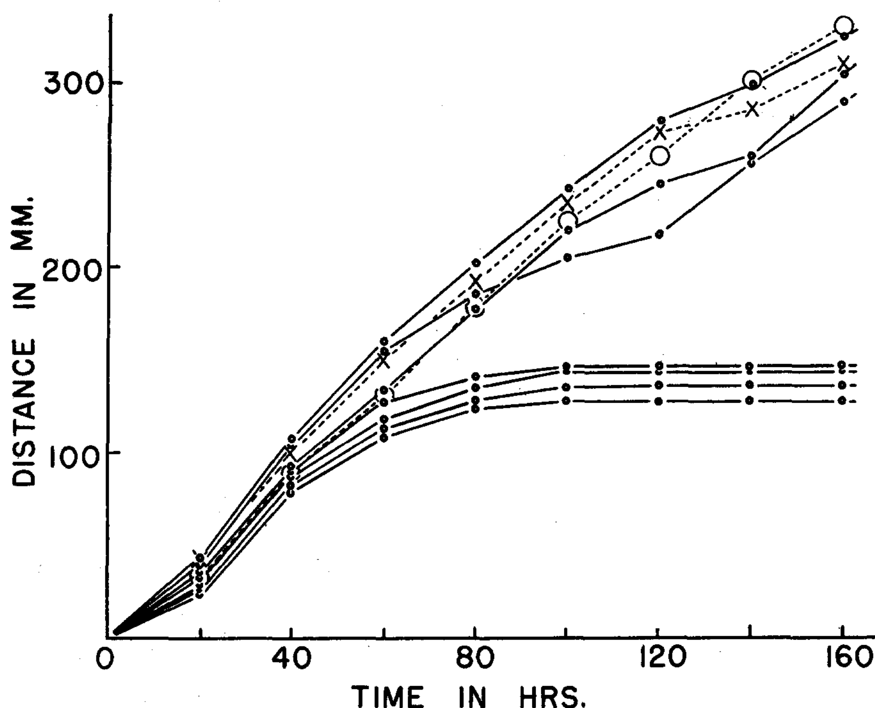

FIGURE 1.—Segregation of *nd* vs. non-*nd* character in an ascus from a cross of *nd* al_2 *A* (3rd transfer of the original mutant culture) to $+^{nd}$ al_2 *a*–15300. Temperature 37.8°C. o = segregants; X = $+^{nd}$ al_2 *A*–15300; ◯ = $+^{nd}$ al_2 *a*–15300.

on slants of the original culture were labelled as the 1st, 2nd, etc. stock transfers and were kept in a refrigerator. All *nd* cultures ceased to grow at the fourth to sixth transfer on slants at 25°C.

The symbols for the mutant genes used in this investigation are as follows: pantothenicless 5531–22 (*pan*), lysineless 4545 (lys_1), albino 4637 (al_1), albino 15300 (al_2), leucineless 33757 (leu_1), p-aminobenzoicless 1633 (*pab*), amycelial 422 (*am*), microconidiating (pe^m), fluffy (*fl*) and natural death (*nd*).

When growth was measured in liquid cultures, 40 ml of Fries No. 3 medium with 1 percent sucrose per 125-ml Erlenmeyer flask was generally adopted. The growth tube method of measuring growth gives a more accurate measurement of instantaneous growth rate and helps one to visualize the limitation of growth and hence was used in most cases (RYAN, BEADLE and TATUM 1943). For measurement of maximal amount of uninterrupted growth at temperatures lower than 32°C, tubes of 62 and 88 cm in length were necessary, because for some unknown reason the total amount of growth was much less if growth was interrupted by subculturing. Short (40 cm) growth tubes with several side arms were used whenever sampling along the tube was desired.

The sorbose plating technique was used whenever discrete colonies were desired (TATUM, BARRATT and CUTTER 1949). Agar with 0.5 percent sorbose plus 0.01 percent sucrose was found to be satisfactory for counting purpose. In attempts to isolate homocaryotic strains, 0.05 percent sorbose plus 0.01 percent sucrose was found to be more suitable.

All test cultures were run in duplicate unless otherwise mentioned.

EXPERIMENTAL RESULTS

Segregation and localization of the nd gene

When an *nd* culture was crossed to a non-*nd* culture a one to one segregation for the *nd* character was observed in each case. In randomly isolated spores a statistical one to one segregation was also observed in each cross.

TABLE 1

The estimation of map distances.

Map distance between	Source of data				Weighted mean	Values from H.B.C.† with no. of whole asci in parentheses
	Randomly isolated spores (172)			Whole asci (24)		
	Cross 1 (67)	Cross 2 (50)	Cross 3 (55)	Cross 4, 5, 6, 7		
Sex to centromere	...	...	...	8.4	8.4	5.8 (1811)
al_2 to centromere	...	...	...	29.0	29.0	24.6 (191)
lys_1 to centromere	...	...	...	...	...	37.5 (64)
sex to al_2	35.4	32.0	30.9	37.4 "42.1"	33.5	30.4*
sex to lys_1	35.0	34.0	...	...	34.6	43.3*
al_2 to lys_1	4.4	18.0	...	...	9.8	12.9*
nd to centromere	...	...	...	14.6	14.6	...
nd to sex	24.6	26.0	14.6	23.0 "20.9"	22.0	...
nd to al_2	21.1	16.4	26.0	14.4 "34.2"	20.5	...

Cross 1: *nd* al_2 $+^{lys_1}$ *a*-F 3 × $+^{nd}$ $+^{al_2}$ lys_1 *A*-4545.
Cross 2: *nd* al_2 lys_1 *a*-64 × $+^{nd}$ $+^{al_2}$ $+^{lys_1}$ *A*-C8.
Cross 3: *nd* al_2 $+^{pe^m}$ $+^{fl}$ *A*-A 1 × $+^{nd}$ $+^{al_2}$ pe^m *fl* *a*-8743-21(13-7).
Cross 4: *nd* al_2 *a*-F 3 × $+^{nd}$ $+^{al_2}$ *A*-crassa 1 A.
Cross 5: *nd* al_2 lys_1 *a*-64 × $+^{nd}$ $+^{al_2}$ $+^{lys_1}$ *A*-C 8.
Cross 6: *nd* al_2 $+^{leu_1}$ *A*-A 1 × $+^{nd}$ $+^{al_2}$ leu_1 *a*-33757.
Cross 7: *nd* al_2 *A* (original) × $+^{nd}$ $+^{al_2}$ *a*-15300.

Numbers in parentheses indicate the number of single spores or asci isolated in each case.
Numbers in quotation marks stand for values estimated from recombination.
* Derived values.
† H.B.C. Houlaham, Beadle and Calhoun 1949.

An analysis of 24 whole asci and 172 randomly isolated spores gives the estimated map units between several genes shown in table 1. The gene is tentatively localized on the right arm of the mating type chromosome (linkage group I) about 15 map units from the centromere.

Dominance relationship

Heterocaryons involving *nd* and non-*nd* components were set up to determine dominance relationship (cf. BEADLF and COONRADT 1944; PONTECORVO 1946; BARRATT and GARNJOBST 1949). The results of such studies are summarized in table 2.

TABLE 2

Growth characteristics of heterocaryons on 40 cm growth tubes with minimal agar at 25° C.

Heterocaryon	Growth rate mm/hr	No. of tubes survived before stop	% of conidia *nd* : non-*nd* : heterocaryotic	Ratio of *nd*/non-*nd*
No. 1*	3.1–3.5	1.5	...	...
No. 2*	4.5	3	...	...
No. 3*	3.8–4.3	2.5	...	...
No. 4*	3.2	1.5	...	...
No. 5*	3.9–5.3	2.5	...	...
No. 6	2.6–3.2	8	32:42:26	0.8/1 (0.7/1–1/1)
No. 7**	3.7–4.2	0.5–1.5	...	...
No. 8†	3.6–4.5	19	49:27:24	1.8/1 (1.2/1–3.5/1)
No. 9	3.3–3.4	6	32:68:0.2	0.5/1 (0.4/1–0.8/1)
No. 10	3.4–4.0	6	24:30:46	0.8/1 (0.4/1–1.3/1)

No. 1: $+^{nd}\ +^{al_2}\ al_1\ +^{leu_1}\ +^{lys_1}\ +^{pan}\ pab\ am\ A$ with $nd\ al_2\ al_1\ leu_1\ +^{pab}\ +^{am}$ A-A 5.

No. 2: The same with $nd\ al_2\ +^{al_1}\ lys_1\ +^{pab}\ +^{am}$ A-C 7.

No. 3: The same with $nd\ +^{al_2}\ al_1\ pan\ +^{pab}\ +^{am}$ A-10.

No. 4: $+^{nd}\ +^{al_2}\ +^{al_1}\ +^{leu_1}\ +^{lys_1}\ +^{pan}\ am\ A$ with $nd\ al_2\ +^{al_1}\ leu_1\ +^{am}$ A-5.

No. 5: The same with $nd\ +^{al_2}\ al_1\ pan\ +^{am}$ A-10.

No. 6: The same with $nd\ al_2\ +^{al_1}\ lys_1\ +^{am}$ A-C 7.

No. 7: $+^{nd}\ al_2\ +^{al_1}\ lys_1\ +^{pan}$ A-D 3 with $nd\ +^{al_2}\ al_1\ +^{lys_1}\ pan$ A-10.

No. 8: $nd\ al_2\ +^{al_1}\ lys_1\ +^{pan}$ A-C 7 with $+^{nd}\ +^{al_2}\ al_1\ +^{lys_1}\ pan$ A-22.

No. 9: $+^{nd}\ +^{al_2}\ +^{lys_1}\ pan\ pe^{m}\ fl$ A-2 with $nd\ al_2\ lys_1\ +^{pan}\ pe^{m}\ +^{fl}$ A-13.

No. 10: $+^{nd}\ +^{al_2}\ al_1\ +^{lys_1}\ leu_1\ +^{pe^m}\ +^{fl}\ A$ with $nd\ al_2\ +^{al_1}\ lys_1\ +^{leu_1}\ pe^{m}\ fl$ A-13.

* There is probably selection for the *nd* nuclei, since the stopped frontiers appear mycelial in all cases.

** There is selection for the non-*nd* nuclei, since there is growth in heterocaryons made between the stopped frontiers and pantothenicless strains but not with lysineless strains.

† In one case a heterocaryon ceased to grow after thirteen transfers without significant change in conidia ratio.

As no *nd* strains and heterocaryons made of two *nd* strains survived more than three transfers in growth tubes at 25°C, those heterocaryons containing *nd* and non-*nd* components that survived six or more transfers with quite constant growth rates are very likely balanced heterocaryons which will survive indefinitely. The ratio of *nd* to non-*nd* homocaryotic conidia of such heterocaryons has been shown to vary from 0.4/1 to 3.5/1 in all cases. The tentative conclusion is therefore that one non-*nd* nucleus is somehow able to overcome the effect of 0.4 to 3.5 *nd* nuclei. Selection of either the *nd* or non-*nd* nuclear component in the heterocaryons was frequently observed. (See notes of table 2.)

Temperature responses

That the *nd* mutant is temperature sensitive is evident from the condition under which this mutant was picked up. The initial growth rate varies with temperature in the same manner as in the albino non-*nd* strain from which it was derived and in a way similar to the wild type *N. crassa* 1 A (RYAN, BEADLE and TATUM 1943). Besides growth rate the maximum amount of growth achievable is also a function of temperature (fig. 2, table 3). This is true not only of growth in tubes but also in liquid cultures. It is striking to see, however, that in liquid cultures the non-*nd* strain gives a set of growth curves similar to that of the *nd* strain (fig. 3). Nevertheless the limiting factors are different. After growth had stopped, bits of mycelium were transferred to new flasks. There was very little growth observed in the case of

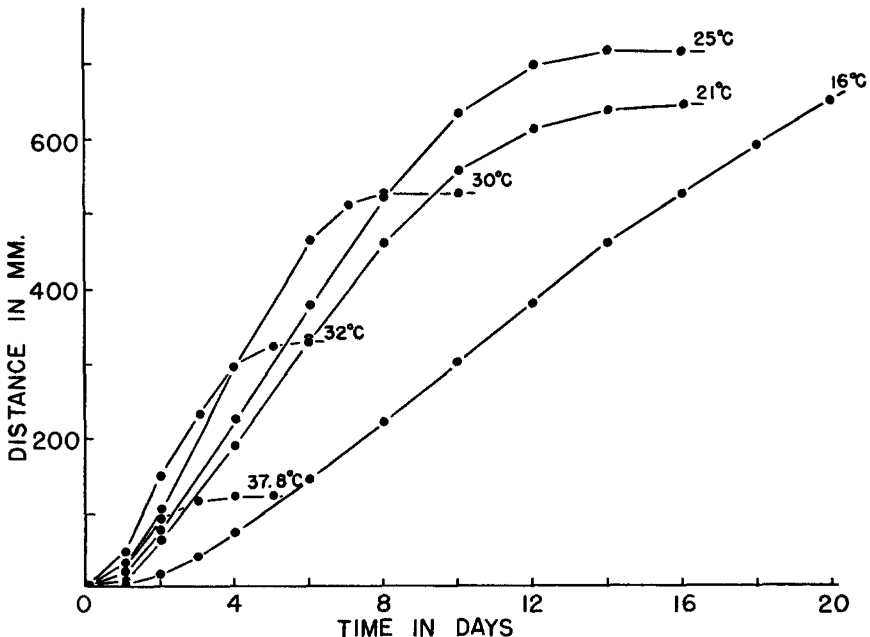

FIGURE 2.—Uninterrupted growth of *nd* al_2 *A*–A 1 (original single-spore culture) in growth tubes at different temperatures.

TABLE 3

Maximum distance reached by nd strains at different temperatures, the corresponding times and their reciprocals. D-Ca-pantothenate concentration 7.5 γ/15 ml for the pantothenicless strain.

Temperature	*nd al_1 pan A*-10 (1st stock transfer)			*nd al_2 A*-A 1 (1st stock transfer)		
	x (mm)	t (hr)	1/t	x (mm)	t (hr)	1/t
37.8°C	38	40	0.0250	46	49	0.0204
32°C	284	118	0.0084	198	121	0.0083
30°C	290	118	0.0084	262	247	0.0041
25°C	462	204	0.0049	434	356	0.0028
21°C	490	312	0.0032	390	344	0.0029
16°C	510	650	0.0015	410	667	0.0015

the *nd* strain, but full growth was observed in every case with the non-*nd* strain.

As t (table 3) is the time when the *nd* strain stops growing, 1/t is an arbitrary measurement of the rate of cessation of growth. When the logarithms of the value of 1/t (in percentage) which are taken from the smoothed curve of 1/t (in percentage) against temperature, are plotted against the re-

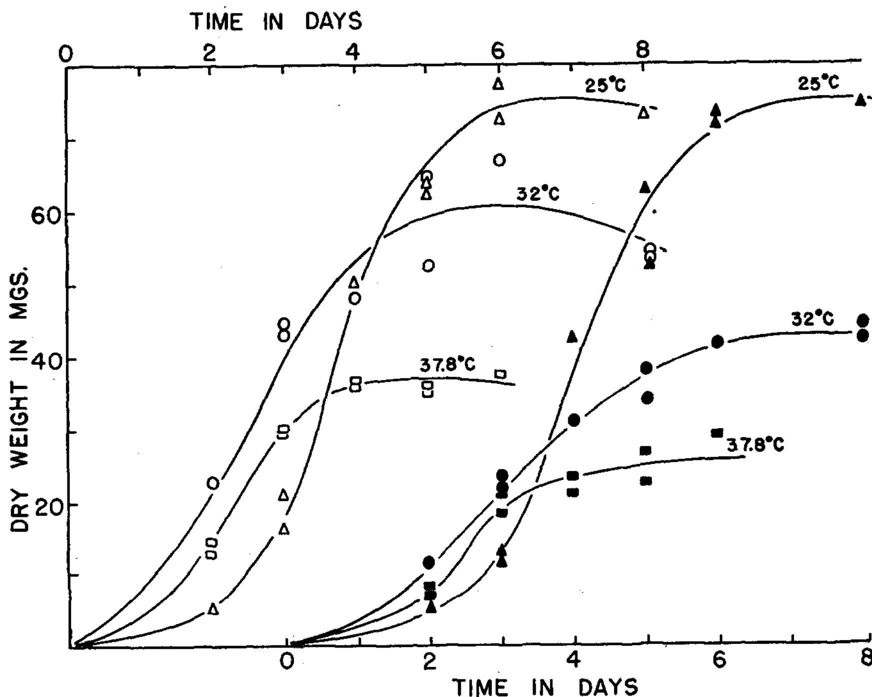

FIGURE 3.—Growth of *nd al_2 A*–A 1 (original single-spore culture) and $+^{nd}$ *al_2 A*–15300 in liquid cultures at different temperatures. Right set: *nd al_2 A*–A 1; left set: $+^{nd}$ *al_2 A*–15300.

ciprocal of absolute temperature (1/T) we have two straight lines intersecting at 33°C for the *nd* al_1 *pan* A–10 strain (fig. 4). The apparent energies of activation are 36,600 and 14,300 cal/mol for the upper and lower sections. A determination on another *nd* strain gives an intersection at 31°C and energies of activation of 38,300 and 16,000 cal/mol. The intersecting temperature seems to mean that below this temperature the cessation of growth is caused by the effect of the *nd* gene; while above this temperature cessation is primarily due to the effect of high temperature on Neurospora protoplasm.

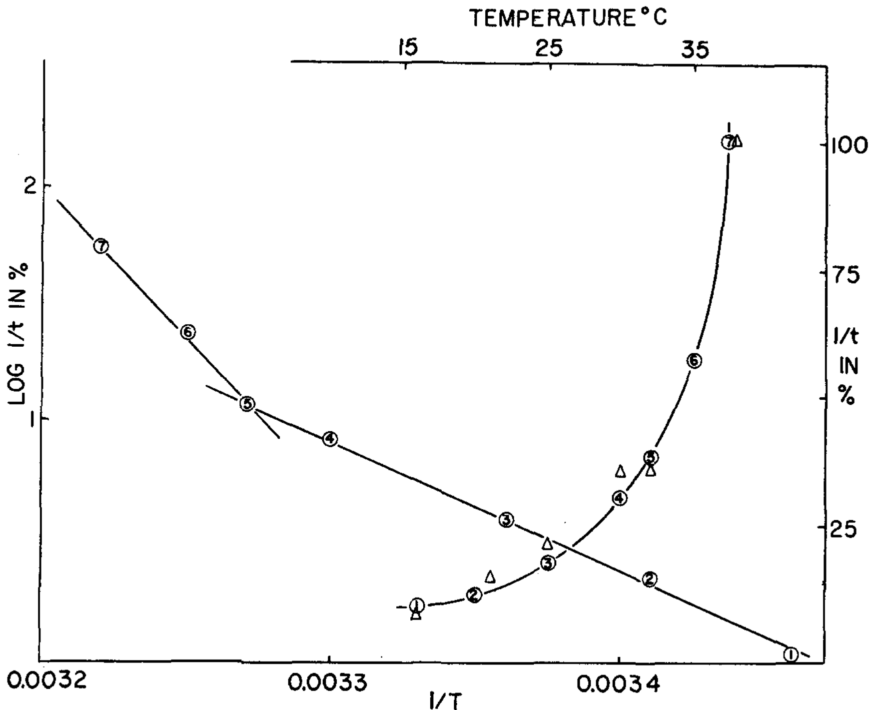

FIGURE 4.—Temperature characteristics of *nd* al_1 *pan* *A*–10. Right curve (upper right coordinates): 1/t in percentage against temperature °C; left curve (lower left coordinates): log 1/t in percentage, using values of 1/t in percentage taken from the right curve against 1/T. △ = experimental points; ○ = values taken from the right smoothed curve.

There are several reasons for this belief. Firstly, the optimum temperature for the rate of growth of the *nd* strain as well as the parental non-*nd* strain corresponds approximately to the intersecting temperature. Secondly, the cessation of growth of the *nd* strains at 32 and 37.8°C was usually temporarily reversed when they were transferred to 25°C. Thirdly, the growth of non-*nd* strains at 37.8°C did not usually occur at an uniform rate (fig. 1) and this was observed in some cases even at 32°C. Furthermore, the cessation of growth of the non-*nd* mold at higher temperature is reversed when the temperature is lowered to 25°C (RYAN *et al.* 1943).

The fact that the value of the apparent energy of activation of the lower section is of the order of ordinary enzymatic or biological reactions while that of the upper section is close to the value of heat inactivation of enzymes may suggest that the self-intoxicating process is not due to the destruction of certain enzymes during growth (SIZER 1943).

Growth rate and longevity

The growth rate of any Neurospora strain is certainly not a function of temperature alone. Different nutritional conditions affect the growth rate considerably. It would then be interesting to see what the relationship of growth rate to the maximal amount and the duration of growth is, if the temperature is kept constant.

The growth of an *nd* strain at 32°C in growth tubes with different concentrations of the salts of Fries medium is shown in figure 5.

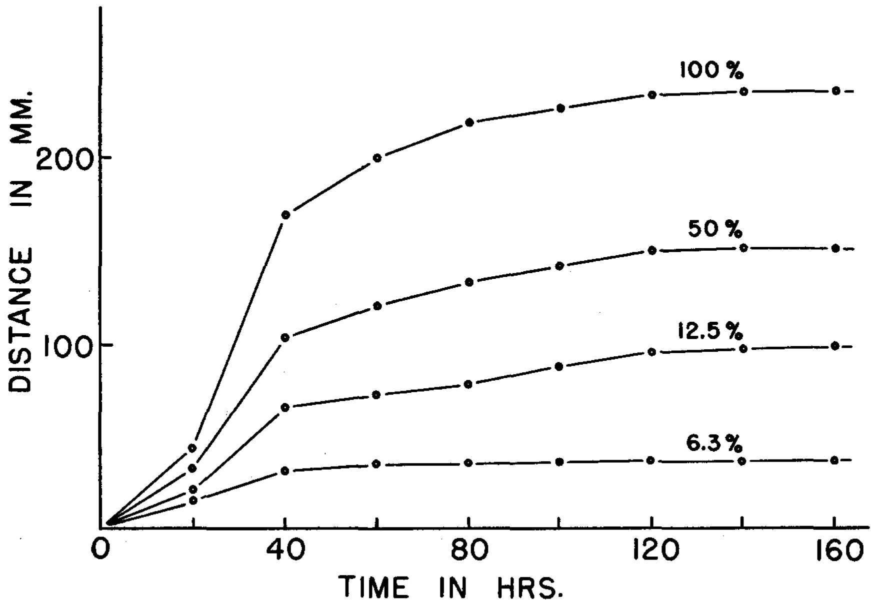

FIGURE 5.—Growth of *nd al₂ A*–A 1 (1st stock transfer) in tubes containing Fries agar of different salt concentrations at 32°C.

In connection with the test for the possible condition under which the *nd* strain might grow indefinitely, media of different composition and supplements were tried. This includes variation in condition of aeration, in salt concentrations, in biotin concentration, in sucrose concentration, in pH from 3.6 to 8.0, in the proportion of Na^+ and Ca^{++}, in media with yeast extract, casein hydrolysate, in Neurospora extract heated to boiling and with individual amino acids and water soluble vitamins placed in front of growing tips of old mycelium. Nothing was found to promote the growth of the *nd* strains considerably beyond that achieved under usual cultural conditions on minimal

medium. It was also observed that just as the results shown in figure 5, different nutritional conditions affect the initial growth rate and the amount of growth very much but less the duration of the growing period.

In passing it should be mentioned that *nd* extract had no injurious effect on the non-*nd* strain under the same experimental conditions.

Ageing under non-growing and other conditions

Experiments were performed to see if there is ageing in non-growing cultures. Transfers were made from the first, second, and the third stock transfers to four sets of 10 x 70 mm tubes labelled as the second, third and fourth stock transfers and let grow at 25°C for three days. A test in growth tubes was then made at 30°C, meanwhile these four sets were kept separately at 12, 25, 32, and 37.8°C for further tests, which were made at the tenth and the twentieth days after the first test. The results in table 4 show that ageing

TABLE 4

Ageing of nongrowing cultures of nd al_2 a-F 3 of different stock transfer (or age, kept at different temperatures as shown by the total amount of growth in mm reached at 30°C.

Stock transfer	Date when test made	Temperature under which stocks were kept			
		12°C	25°C	32°C	37.8°C
	1st day	257	265	301	304
2nd stock	10th day	202	243	186	186
transfer	20th day	211	264	225	8
	1st day	165	159	192	164
3rd stock	10th day	147	154	142	110
transfer	20th day	149	258	217	21
	1st day	32	58	91	12
4th stock	10th day	24	25	59	20
transfer	20th day	39	15	8	0

is going on in the non-growing cultures too. The process of ageing is however much slower compared with ageing during growth.

It has been mentioned that for some unknown reason the growth potential decreases tremendously under conditions of repeated subculturing. Table 5 illustrates the results of an experiment using growth tubes with side arms for taking samples along the tube. In the first column the figures stand for the distances from the inocula to the points where subcultures were taken. In the second column the distances reached by the subcultures in separate growth tubes are recorded. Sample A was taken at about 1 cm behind the growing frontier and sample B taken after the growth had stopped at the end of the tube which was 329 mm from the beginning. The values of the third column therefore represent the expected distance if the mold were not subcultured, and the final column the percentage of distance realized taking the values in the third column as one hundred percent.

TABLE 5

Effect of subculturing on the growth of nd al_2 A-A 1 1st stock transfer at 32° C.

Subculture taken from	Distance grown in mm after subculturing		Expected distance if not subcultured	Percentage realized	
	Sample A*	Sample B*		Sample A	Sample B
48 mm	120	61	329 - 48 = 281	43	22
96 mm	62	35	329 - 96 = 233	27	15
150 mm	24	20	329 - 150 = 179	13	11
199 mm	19	0	329 - 199 = 130	15	0
252 mm	3	0	329 - 252 = 77	4	0

* See text.

Rejuvenation through heterocaryosis

It has been shown that balanced heterocaryons can be established in certain cases. It is therefore suggested that if the ageing process were due to the accumulation of some toxic substances, or the exhaustion of some essential substances, one would naturally expect that the *nd* strain might be rejuvenated if pure strains were isolated from those balanced heterocaryons which show no cessation of growth. For this purpose heterocaryons between microconidiating cultures *nd* al_2 lys_1 $+^{pan}$ pe^m *fl* A–13 and $+^{nd}$ $+^{al_2}$ $+^{lys_1}$ *pan* pe^m *fl* A–2 (No. 9, table 2) were used. Experiments confirmed the findings of BARRATT and GARNJOBST (1949), that there is at most 0.5 percent of heterocaryotic microconidia which give rise to colonies on minimal agar plates. Conidia were plated on agar with 0.05 percent sorbose and 0.01 percent sucrose supplemented with an optimal concentration of DL-lysine (0.5 mg/15 ml) or (+)-Ca-pantothenate (7.5γ/15 ml) for the two types of homocaryotic colonies. Individual colonies were transferred directly to growth tubes supplemented with optimal lysine. Conidia from pure cultures of the non-*nd* strain and the first stock transfer of the *nd* strain were also plated and transferred parallel to the experimentals. Conidia are so few and may be so inviable in the third stock transfer of the *nd* strain (practically none are present in the fifth stock transfer) that corresponding controls were taken directly from the pure cultures instead of from single conidial colonies. Ten colonies were tested in each case. The results are shown in table 6.

The results are striking enough to show that no matter how " old " the *nd* strain is, it will rejuvenate through heterocaryon formation to about the same extent. All *nd* isolates from heterocaryons exceed the corresponding pure cultures in the amount of growth and approach or equal that reached by the original culture, which under the same conditions gave 552 mm of growth in two determinations. The corresponding non-*nd* (and *pan*) isolates were tested in growth tubes supplemented with optimal Ca-pantothenate. The means of the growth rates at 30°C are 4.1 ± 0.4, 4.1 ± 0.3 and 4.0 ± 0.3 mm/hr for non-*nd* isolates from heterocaryons made between the first and the fifth stock transfer and a non-*nd* culture, and from the non-*nd* culture

TABLE 6

Rejuvenation through heterocaryosis. Total amount of growth in mm reached by nd isolates from balanced heterocaryons made between different stock transfers of $nd\ al_2\ lys_1\ +^{pan}\ pe^m\ fl$ *A-13 and* $+^{nd}\ +^{al_2}\ +^{lys_1}\ pan\ pe^m\ fl$ *A-2 and corresponding colonies or inocula from pure cultures of the nd component.*

nd (and lys_1) colonies or inocular from	1st stock transfer		3rd stock transfer		5th stock transfer	
	Colonies from hetero-caryon	Colonies from pure culture	Colonies from hetero-caryon	Inocula from pure culture	Colonies from hetero-caryon	Inocula from pure culture
Approximate total amount of growth in mm at 32°C	380	305	440	250	605	0
	455	325	605	260	670	0
	390	350	375	250	600	...
	410	325	410	250	495	...
	370	140	620	...	510	...
	305	580	500	...	685	...
	605	535	615	...	470	...
	630	0	665	...	255	...
	605	55	300	...	600	...
	0	324	0	...	400	...
Mean (disregarding zero readings)	461	380	505	252	529	0

itself. It is, however, a peculiarity of this non-*nd* culture that even at temperatures as low as 30°C the growth rate is as a rule not constant.

Rejuvenation through crossing

Similar experiments were done using the sexual process instead of asexual heterocaryon formation. Crosses were made between the different stock transfers (or ages) of the same strain $nd\ al_2\ a$–F 3, and a single non-*nd* strain, *N. crassa* 1 A. Ascospores were isolated, activated and tested in growth tubes. The results were analogous to those in the experiments with heterocaryons, *i.e.*, all of the *nd* segregants were rejuvenated to the same extent no matter what growth phase or which stock transfer was used in the cross.

From the experiments on rejuvenation through heterocaryosis and through crossing it seems reasonable to suspect that there might be a difference between reciprocal crosses. Crosses were therefore made using one or the other strain as the protoperithecial parent or the conidial parent. Spores were isolated and tested and all the *nd* segregants were found to be rejuvenated to the same extent. The negative result may be due to the fact that the difference in the amount of cytoplasm contributed by the conidium and by the trichogyne is not large enough to be demonstrated by reciprocal crossing.

It was thought that if the cytoplasm of the non-*nd* parent that had been used in these crosses was responsible for the rejuvenation, a cross of two aged *nd* strains would not be expected to yield rejuvenated spores. Repeated trials to cross *nd* to *nd,* old and young, using Westergaard's agar, plain or

supplemented with yeast extract and casein hydrolysate, corn meal agar, normal and very low concentration of sucrose (0.01 percent) and at different temperatures, always led to failure. A cross was also made between *nd* al_2 a–49 and a heterocaryon consisting of the amycelial strain $+^{nd}+^{al_2}+^{lys_1}$ *am* A–422–6 and *nd* al_2 lys_1 $+^{am}$ A–C 7. Five asci were isolated which gave five to eight germinated spores per ascus upon activation. All of them proved to be *nd* and grew as long as the parental cultures. Crosses with heterocaryons were therefore successful and *nd* nuclei of different mating types were shown to be able to undergo syngamy and meiosis.

DISCUSSION

A discussion of the nature of the *nd* mutant is necessarily based on indirect evidence as described above.

Evidence such as rejuvenation through heterocaryosis and crossing between two *nd* strains if one of them is in the heterocaryotic condition leads one to the belief that ageing is going on in the cytoplasm.

A number of hypotheses may be proposed to explain this ageing process and rejuvenation through heterocaryosis as well as crossing. Something injurious might be accumulated during growth, in this case rejuvenation would mean the dilution or destruction of the toxic substance. Something essential might be exhausted during growth, in this case rejuvenation would mean the introduction of this substance into the *nd* ascospore or conidium.

In liquid medium we get about 80 mg of mycelium in each culture inoculated from the original single-spore culture. A calculation of the total amount of mycelium obtainable from such a single-spore culture and the amount of substance that might be carried along in an ascospore or conidium suggests that the later hypothesis is less likely. It requires the substance to be at least a hundred times as active as biotin to support that amount of growth (MELIN and NORKRANS 1948).

The hypothesis of a defective enzyme is also less acceptable; if so it seems that the mutant would grow at a lower but uniform rate instead of high initial but ever-decreasing rate.

The hypothesis of the accumulation of a self-intoxicating substance is more likely though there is no direct experimental support. Besides rejuvenation it explains the temperature response by assuming that temperature affects the rate of synthesis of the *nd* substance more than the rest of the anabolic activities, consequently the higher the temperature the smaller the amount of growth.

There still remain some unexplained facts. It seems that different nutritional conditions affect reactions determining the rate of growth but leaving the synthesis of the *nd* substance relatively unaffected so that we have a picture like figure 5 when the *nd* strain is subject to different nutritional conditions. The whole story is of course complicated by the very complex mode of growth (RYAN *et al.* 1943) by which any substance may accumulate in the growing frontier of the mycelium. Facts like the tremendous decrease in

growth potential after subculturing are entirely unexplained. The conclusion of an accumulation of self-intoxicating substance is therefore only tentative until the substance is isolated or at least its action demonstrated in vitro.

SUMMARY

A mutant strain, called natural death or *nd*, was induced in *Neurospora crassa* by ultraviolet radiation and isolated by means of the sorbose plating technique. It is characterized by its ever-decreasing growth potential under all nutritional conditions and the irreversible cessation of growth either on agar surface or in liquid culture. The responsible gene has been localized on the right arm of the mating type chromosome about 15 map units from the centromere. The *nd* gene is recessive to its wild allele, because balanced heterocaryons that seem to grow indefinitely are formed with non-*nd* strains and the ratio of *nd* to non-*nd* conidia is not far from unity.

The mutant is temperature-sensitive in the sense that the higher the temperature, the higher the growth rate and lower the amount of growth. However, if the temperature is kept constant and the growth rate altered by changing the nutritional condition, then the higher the growth rate, the higher the amount of growth. Ageing not only goes on during growth but also under non-growing conditions.

The aged strain can be rejuvenated through heterocaryosis with non-*nd* strains, through crossing to non-*nd* strains, and through crossing to another *nd* strain which is in a heterocaryotic condition. In each case the degree of rejuvenation is independent of the age of the *nd* strain used. Direct crossing of one *nd* strain to another has not yet been possible.

A hypothesis of cytoplasmic ageing, assuming the accumulation of a self-intoxicating substance in the growing frontier is proposed.

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