INVESTIGATIONS OF SOME "UNKNOWN" MUTANTS OF *NEUROSPORA CRASSA*

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ABSTRACT

A sample of 16 previously "unknown" mutants of Neurospora crassa was studied. Five of these were shown to be double mutants. The growth requirements of 19 of the 21 single-gene mutants available then from these "unknowns" were at least partially defined chemically. Of these 19, three have been classified as serineless, two as asparagine-less, two as pyrimidineless, two as succinicless, and one each as aceticless, arginineless, aromaticless, asparticless, homoserineless, leucineless, methionineless, methionine-adenine-cystineless, p-amino-benzoicless, and slow-grower. These had been classified as "unknowns" apparently for various reasons, such as: (1) inhibitions from normal metabolites, (2) complex requirements for double-mutant "unknowns," and (3) absence of active compound from chemically defined mixtures previously used. Some of these mutant genes have been located genetically. These results have been discussed briefly with reference to the hypothesis of the "unifunctionality" of the gene.

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I. INTRODUCTION

The commonly studied biochemical mutants of Neurospora crassa are those which will grow better on one or another "complete" medium than they will on minimal medium (Beadle and Tatum, 1945). Horowitz and Leupold (1951) have called these mutants "dispensable." The "unknowns" are those "dispensable" mutants whose requirements for growth have not been chemically defined. These "unknowns" are usually classified as such after preliminary investigations have shown that some likely known compounds and some likely known mixtures of compounds are ineffective in stimulating growth.

Within this group of mutants one could expect to find many of unusual interest. Among these might be mutants which require a new vitamin, mutants with new blocks in carbohydrate metabolism, mutants with a genetic inability in peptide bond synthesis, mutants useful in further investigation of amino acid metabolism, and other useful ones. The possibility of finding such interesting types was one reason for undertaking this work.

A second reason for this investigation relates to the work of Horowitz and Leupold (1951). Many mutants of Neurospora crassa will grow maximally or nearly maximally when the minimal medium is supplemented with a single compound. Horowitz and Leupold have called the wild-type allele of the mutant gene in such a strain "unifunctional." In arriving at a minimal estimate of the proportion of "unifunctional" genes among the genes which mutate lethally, these workers have assumed that all of the "unknowns" possess lethal alleles of "multifunctional"

genes. The "unknowns" investigated in the work reported in this thesis constitute a more or less random sample of all the "unknown" mutants. The results obtained here thus affect the estimate made by Horowitz and Leupold.

One of the earliest studies of an "unknown" mutant of Neurospora crassa was made in 1943 by Bonner, Tatum, and Beadle. They investigated strain 16117. Preliminary work had shown that this mutant would grow on acid-hydrolyzed casein but not on the synthetic mixtures of amino acids tried nor on any other known mixtures of compounds tried. These investigators obtained an active crystalline preparation from acid-hydrolyzed casein and showed that this was a mixture of 60% l-isoleucine, 25% l-leucine, and 15% l-valine. They demonstrated that maximal growth of 16117 could be obtained on l-isoleucine plus l-valine and that l-leucine had a sparing effect on the l-valine requirement. The inactivity of their synthetic amino acid mixtures which contained valine and isoleucine was ascribable to the phenylalanine, norleucine, and norvaline also present. These three amino acids inhibit the growth of 16117.

Mauron (1950) conducted an investigation of some "unknown" mutants of Neurospora crassa. In particular he elucidated the growth requirements of a previously "unknown" strain with the occurrence number 44601. He found that this would grow nearly maximally if supplied with both adenine and arginine. If the minimal medium was supplemented with these two compounds and threonine, growth was yet nearer maximal. Several other purines and pyrimidines would substitute at least partially for the adenine requirement. Maximal growth was obtained, however, only

if arginine and an unknown factor in commercial peptone were supplied. This unknown substance was purified but not characterized. From an outcross of mutant 44601 to wild type, 21 asci, each of which had at least three spore-pairs represented, were classified (Houlahan, Beadle, and Calhoun, 1949). None had more than two mutant spore-pairs. It is likely then that there are not two unlinked mutant genes for biochemical deficiencies in mutant 44601.

Markert (1952), working with "dispensable" biochemical mutants of Glomerella, found it much easier to define chemically the nutritional needs of those with absolute requirements for some nutrilite than of those which grew slowly on minimal medium. For some of these latter partial nutritional mutants he reported rather nonspecific stimulations by vitamin mixtures and he considered these mutants to have several synthetic abilities diminished as a result of the single mutation. In the work reported here the "unknown" strains, as a group, have about as absolute requirements for growth as does a representative sample of mutants for which the nutritional requirements are known.

II. MATERIALS, METHODS, AND NOMENCLATURE

Mauron and Dubes (1950) made a search for "unknown" mutants produced through ultraviolet irradiation. Mutants were classified as "unknown" if they failed to grow on minimal medium (Beadle and Tatum, 1945) supplemented with certain combinations of known chemical compounds but grew on at least one of the media with complex supplementation. Four media with chemically known, or largely known, supplementation were used. These had the following designations and compositions: (A) acid-hydrolyzed casein and tryptophane; (M-4) methionine, lysine, tryptophane, adenine, cytidine, and succinic acid; (V) thiamine, riboflavin, pyridoxine, pantothenic acid, p-aminobenzoic acid, nicotinamide, choline, inositol, and folic acid; (V-4) riboflavin, folic acid, vitamin B12, and biocytin. The three complex media used had the following designations and supplementations: (P) "Bacto-Peptone" (Difco); (L) a charcoal-adsorbed aniline-eluted fraction of liver extract (Lilly); (C) an autolysate of wild-type Neurospora mycelium, acid-hydrolyzed casein, and tryptophane (Lein, Mitchell, and Houlahan, 1948). The growth tests were performed at 25° C. Thirteen mutants, "unknown" by the above criteria, were obtained. Further investigations showed that only four of these were clear-cut "unknowns"; the remainder proved either to have reverted to wild type, to be indispensable slow-growers, or to grow on a medium supplemented with all four of the above-mentioned mixtures of known compounds. These four "unknowns" were given preliminary occurrence designations of circle-16, alpha-10, alpha-51, and delta-19.

Over the years of work with Neurospora at the California Institute of Technology and at Stanford University mutants have been found and classified as "unknown" by various investigators. Many of these are still in the stocks at the California Institute of Technology. It was from this group that Dr. Herschel K. Mitchell selected twelve apparently promising "unknowns." These have occurrence designations of 35709, 36111, 37812, 44303T, 45208T, 46005, 46403, 47903, 65004, 65108T, 67603T, and 83901T. All of these except 83901T came from cultures which had been treated with ultraviolet light. Irradiation data on these were given by Beadle and Tatum (1945). Mutant 83901T came from a culture subjected to X-rays at a dosage of 100,000 roentgen units (M. B. Mitchell, 1952).

Further research with these twelve and with circle-16, alpha-10, alpha-51, and delta-19 is reported in this thesis.

In general, the methods used have been standard ones for work in Neurospora biochemical genetics (Beadle and Tatum, 1945). The quantitative growth experiments were performed in 20 ml. of minimal medium, unsupplemented or supplemented appropriately, in 125-ml. Erlenmeyer flasks. The media were sterilized by autoclaving. Inoculations were made from dilute suspensions of conidia and/or mycelial strands in sterile distilled water. These growth trials were usually run at 25° C., but in special cases at 36° C. The duration of the growth period varied from about three to five days, depending especially on the strain being investigated. Masses of mycelia were determined after drying at about 90° C. for two or more hours.

Growth experiments with the "unknowns" were performed on various

types of media, both chemically defined and undefined. The various media used, with their symbols, include the following:

Defined

M: minimal medium

V: choline chloride (2 γ/ml.)
folic acid (0.00004γ/ml.)
i-inositol (4 γ/ml.)
nicotinamide (2 γ/ml.)
calcium pantothenate (2 γ/ml.)
p-aminobenzoic acid (0.5 γ/ml.)
pyridoxine hydrochloride (0.5 γ/ml.)
riboflavin (0.5 γ/ml.)
thiamine hydrochloride (1 γ/ml.)

V-1: V plus thymine at 0.04 mg./ml.

A: acid-hydrolyzed casein (Nutritional Biochemicals Corp.) (0.5 mg./ml.)
l-tryptophane (0.05 mg./ml.)

M-4: dl-methionine (0.1 mg./ml.)
dl-lysine monohydrochloride (0.25 mg./ml.)
l-tryptophane (0.05 mg./ml.)
adenine sulfate (0.04 mg./ml.)
succinic acid (0.05 mg./ml.)
cytidine sulfate (0.04 mg./ml.)

M-5: the same as M-4 except that cytosine at 0.04 mg./ml. is substituted for the cytidine sulfate.

M-6: M-4 plus cytosine at 0.04 mg./ml.

Vr-1: folic acid (0.01 γ/ml.)

folinic acid (50% pure preparation at 0.02 γ/ml.)

vitamin B12 (0.001 γ/ml.)

biocytin (0.001 γ/ml.)

lyxoflavin concentrate (Merck swine hearts extractives, 0.005 ml./ml.)

p-hydroxybenzoic acid (0.5 γ/ml.)

Vr-2: the same as Vr-1 except that pure lyxoflavin at 0.125 γ/ml. is substituted for the lyxoflavin concentrate.

Vr-3: Vr-2 plus the following:

l-arginine monohydrochloride (0.05 mg./ml.)

l-asparagine (0.05 mg./ml.)

Vr-4: Vr-1 plus the following:

l-arginine monohydrochloride (0.05 mg./ml.)

l-asparagine (0.05 mg./ml.)

Ex-1: l-histidine monohydrochloride (0.025 mg./ml.)

thymidine (0.02 mg./ml.)

uridine (0.02 mg./ml.)

guanosine (0.02 mg./ml.)

(-)-quinic acid (0.025 mg./ml.)

shikimic acid (0.008 mg./ml.)

Ex-2: the same as Ex-1 except that thymine at 0.04 mg./ml. is substituted for shikimic acid and the concentrations of all the others are doubled.

Ex-3: the same as Ex-2 except that uridine is omitted.

Various combinations of these, indicated by symbol combinations; for example, A Vr-1 is a combination of mixture A and mixture Vr-1.

Undefined

C: autolysate of Neurospora (ca. 2 mg./ml.)
acid-hydrolyzed casein (Nutritional Biochemicals Corp.) (0.25 mg./ml.)
l-tryptophane (0.05 mg./ml.)

E: enzymatically hydrolyzed casein (digested with pancreatin; Nutritional Biochemicals Corp.) (2 mg./ml.)

Lv: liver extract (Lilly) (2 mg./ml.)

Ma: malt extract (Difco) (2 mg./ml.)

N: autolysate of Neurospora (ca. 2 mg./ml.)

P: "Bacto-Peptone" (Difco) (2 mg./ml.)

Y: yeast extract (Fleischmann type 300) (2 mg./ml.)

If significant growth was obtained on any of the media with chemically known supplementation, the obvious breakdown experiments were performed. If, however, significant growth was obtained only on media with chemically undefined supplementation, then resolution of the crude growth source was undertaken. Rather early in this work it became apparent that it was worth while to test the strains on all the compounds present in the known mixtures individually before resorting to a resolution of the best crude growth source for the strain. This is necessary since a known mixture might very well contain an inhibitor or inhibitors in addition to the compound or compounds necessary for growth (Bonner, Tatum, and Beadle, 1943; Srb, 1947). In addition to this, known compounds other than the ones listed in the mixtures above were often tried.

Several standard techniques for the resolution of a crude mixture

were used. The most important was that of paper chromatography. Chromatostrips, chromatosheets, and chromatopiles (Mitchell and Haskins, 1949) were used.

The genetic techniques used are common in Neurospora work. Crosses were made on the Westergaard-Mitchell medium (Westergaard and Mitchell, 1947), unsupplemented or supplemented appropriately. Simultaneous inoculations of the two parental strains were not used. Rather, one was allowed to grow and to form protoperithecia, if it would. Conidia and/or mycelial strands of the other parent were then introduced into the tube or Petri plate. Usually the medium was supplemented only with the compound or compounds required by the protoperithecial parent. Reciprocal crosses often gave markedly different fertilities: hence in many instances crosses were attempted in both ways.

Asci were dissected in the usual fashion with a thin glass needle and usually on a plate of 4% agar minimal medium. The spores were activated at 60° C. for ca. 40 minutes. They were classified on the plate, if possible; if not, the spores were transferred to tubes and the classifications were made later. Random ascospores were dispersed over a plate and activated. Classifications were made on the plate or later, as above.

Houlahan, Beadle, and Calhoun (1949) have presented important linkage data for Neurospora crassa. Most of the marker genes used in this study for the location of some of the mutant genes in the "unknowns" were placed by them as to linkage group and centromere distance.

In this paper the strains, cultures, or clones will be referred to by their occurrence designation plus their reisolate designation, if

necessary and available, plus their mating-types. In the case of 45208T-9B-A, 45208T is the occurrence designation, 9B the reisolate designation, and A the mating-type. The T indicates that the mutant is particularly temperature-sensitive. The nutritional genes themselves will be symbolized by an abbreviation of the substance or substances needed for growth with the occurrence designation as a superscript. The mutant gene then in the leucineless strain 45208T-9B-A is leu^{45208T}. Any subscripts used will refer to genetic locus.

When these "unknown" strains proved to be double mutants, a second occurrence designation was needed. For these cases a new designation was assigned to one of the component single-gene mutants; the other one retained the original. New occurrence designations were, however, assigned to all of the single-gene mutants coming from circle-16, alpha-10, alpha-51, and delta-19. These four designations were simply preliminary designations and have been discarded.

III. RESULTS

A. Mutant Circle-16 (C126 and C127)

Genetics

Random spores from the outcross WP1400-4-A (wild-type) x circle-16-a were classified on a plate of minimal agar. The percentage germination was 98. Of the 413 germinated spores classified, 60% were mutant, 17% were intermediate, and 23% were wild type. (The classification was difficult because of these intermediate types.) These results indicated that circle-16-a is mutant for more than one gene.

From this same outcross to wild type, 21 asci were dissected. A classification of these on a plate of minimal agar indicated that some contained more than two mutant spore-pairs. These germinated asci were transferred to tubes for further classification.

The original mutant circle-16-a grows well on cytidine plus serine. These compounds were therefore tested on the reisolates. A classification of these reisolates has been made on the assumption that the original circle-16-a is the double mutant pyrimidineless serineless (Table 1).

The utilization of dl-serine by these serineless reisolates varies considerably. No utilization of formic acid by the two reisolates tested is apparent (Table 2); yet, under different conditions and by other reisolates, it may be utilized.

All of the pyrimidineless reisolates, with the exception of 7A, grow maximally when supplied with cytidine sulfate. Reisolate 7A is by inference a pyrimidineless mutant, but it will grow only very slowly when supplied with cytidine sulfate. The genotype of reisolate 15B

Table 1. Progeny from WP1400-4-A (wild-type) x circle-16-a

		Genotype			
Perithecium No.	Ascus No.	Pair A	Pair B	Pair C	Pair D
1	1	$_{+}pyr_{+}ser$	$_{+}pyr_{+}ser$	died	died
1	5	$pyr^{+}ser$	$pyr^{+}ser$	$_{+}pyr_{ser}$	$_{+}pyr_{ser}$
2	6	$_{+}pyr_{+}ser$	$pyr^{+}ser$	died	$_{+}pyr_{ser}$
2	7	?	$_{+}pyr_{+}ser$	$_{+}pyr_{ser}$	pyr ser
4	15	$pyr^{+}ser$	?	$_{+}pyr_{ser}$	X
4	18	$pyr^{+}ser$	pyr ser	X	X
4	21	$_{+}pyr_{ser}$	died	$pyr^{+}ser$	$_{+}pyr_{ser}$

Key to symbols: pyr = pyrimidineless; ser = serineless; X = failed to germinate; ? = grows only very slowly when supplied with cytidine plus serine.

Table 2. Growth of reisolates with the serineless gene and of the original circle-16-a for 136 hours at 25° C.

Inoculum	Presumed Genotype	Mycelial Mass (mgs.) Medium M	s	f	c	c+s	c+f	N
reisolate 5C	$+^{pyr}$ser	4.2	16.4	3.8	--	--	--	--
reisolate 5D	$+^{pyr}$ser	0.0	trace	--	--	trace	--	6.2
reisolate 6D	$+^{pyr}$ser	trace	6.2	--	--	--	--	--
reisolate 7C	$+^{pyr}$ser	2.1	5.6	2.4	--	--	--	--
reisolate 7D	pyr ser	--	--	--	3.1	26.4	--	--
reisolate 15C	$+^{pyr}$ser	8.1	32.0	--	--	--	--	--
reisolate 18B	pyr ser	--	--	--	3.4	22.7	--	--
reisolate 21A	$+^{pyr}$ser	5.1	38.5	--	--	--	--	--
reisolate 21D	$+^{pyr}$ser	66.2	82.5	--	--	--	--	--
circle-16-a	pyr ser	0.0	--	--	trace	33.9	2.8	--

Reisolates are designated by the ascus number and the pair letter (from Table 1). Trace growth is of the order of magnitude of 0.05 to 0.3 mg. mycelial mass. The symbols are defined as follows: s = dl-serine at 0.1 mg./ml.; c = cytidine sulfate at 0.04 mg./ml.; f = formic acid at 0.1 mg./ml. (added before autoclaving).

cannot be inferred from the data for the rest of ascus number 15.

Reisolate 21A (serineless) has been given the new occurrence designation of C127, and reisolate 21C (pyrimidineless) has been given the occurrence designation C126.

The possibility that the original circle-16-a is a triple mutant has not been excluded. This, however, seems unlikely in view of the random spore data summarized above.

Biochemistry

As mentioned above, the original circle-16-a grows well on cytidine sulfate plus dl-serine (see Table 2). Trace growth can be obtained on cytidine sulfate or uridine alone, but not on dl-serine alone. The compounds adenine sulfate, l-tryptophane, and choline chloride, when supplied in addition to cytidine sulfate, have been observed to stimulate growth beyond the level on cytidine sulfate alone. Table 3 presents growth data for tests of adenine sulfate, cytidine sulfate, l-tryptophane, dl-serine, and all combinations thereof.

The serine requirement of circle-16-a is not replaceable with acid-hydrolyzed casein, although the latter is slightly stimulatory. Table 2 shows that formic acid, in combination with cytidine sulfate, is stimulatory for the growth of the original circle-16-a. Formic acid tested alone, however, is inactive for this original culture.

Of those examined, Neurospora autolysate is the richest chemically undefined source for the growth of circle-16-a.

Table 3. Growth of circle-16-a for 100 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	0.0
a	0.0
c	0.4
t	0.0
s	0.0
a + c	0.5
a + t	0.0
a + s	0.0
c + t	2.1
c + s	31.3
t + s	0.0
a + c + t	0.7
a + c + s	23.0
a + t + s	0.0
c + t + s	31.5
a + c + t + s	24.8
N	50.4

Key to symbols: a = adenine sulfate at 0.04 mg./ml.; c = cytidine sulfate at 0.04 mg./ml.; t = l-tryptophane at 0.05 mg./ml.; s = dl-serine at 0.1 mg./ml.

B. Mutant Alpha-10 (C120)

Genetics

None of the asci listed in Table 4 showed more than two mutant spore-pairs. It is assumed then that this is a single-gene mutant or mutant for two linked genes. The new occurrence designation C120 replaces the preliminary designation alpha-10. The mutant gene will be referred to as *aro* (*aromaticless*). Its centromere distance is four (Table 4).

Table 4. Outcrosses of C120 to wild-type strains

Cross	Percentage of Dissected Asci Classifiable	Number of Classifiable Asci	Number of Asci Segregating II for aro^{C120}
5297-a x C120-A	37	11	1
WP1400-4-A x C120-D2-a	87	27	3
WP1400-4-A x C120-D4-a	82	9	0

Protoperithecial parent is listed first.

Crosses of C120 to several marker genes were made in an attempt to locate aro^{C120}. Random ascospores from these crosses were classified on plates of minimal agar medium. The results are given in Table 5.

The information on the marker genes comes largely from Houlahan, Beadle, and Calhoun (1949). The marker *scumbo* is a visible one difficult to classify on a plate. The centromere distance of *sfo* is from Emerson (1952) and is based on a large number of asci (more than 200). The centromere distance of for^{C24} was obtained by Gershowitz (1952) and is based on 42 asci. The demonstration that for^{C24} is probably independent

Table 5. Progeny from crosses of C120 to marker genes

Cross	Marker Gene Its Linkage Group and Centromere Distance	Percentage Germination	Number of Germinated Spores Classified	Percentage Classified as Wild-Type	Classifiability
C120-A x 46403-a	suc; A; 16	75	263	25	good
C120-A x 3254-H3-a	ad; B; 0.56	75	211	26	poor
C120-A x 5801-R2-a	scumbo; C; 1.2	81	283	19	poor
C120-A x 10575-a	tryp; C; 15.2	78	925	26	good
C120-D2-a x 263-R1-a	pyr; D; 5.72	21	116	22	poor
C120-A x 33933-P1-a	lys; E; 4.8	81	345	24	good
C120-A x 16117-a	isol-val; E; 8.9	84	918	19	good
C120-D4-a x E15172-16014-A	sfo; F; less than 1	95	369	20	fair
C120-A x C24-a	for; not A, B, C,D, or E; 9.6	86	401	24	good

Protoperithecial parent is listed first.

of linkage groups A through E is credited to M. B. Mitchell and Pittenger (1952). The gene symbol for (formicless) indicates a utilization of formic acid. The agar plates used for classification were minimal and the incubation periods for growth were at 25° C. in every case except that of the cross to sfo^{E15172}. For this, minimal plates supplemented with p-aminobenzoic acid at 137 γ/ml. were incubated for growth at 36° C. in order to inhibit growth of progeny with the sfo^{E15172} gene. The data from the cross to the marker sfo were obtained from a single perithecium, the only fertile one of many large black perithecia produced in the cross.

The results presented in Table 5 would seem to exclude aro^{C120} from linkage in groups A, B, C, D, E, F, and the group containing for^{C24}. The data, however, are not very adequate from the crosses to the markers in group B (ad^{3254}) and group D (pyr^{263}), for the reasons of poor classifiability and poor germination, respectively. If, however, aro^{C120} is independent of all the above-mentioned groups, then the logical conclusion is that for^{C24} is in linkage group F and that aro^{C120} is in linkage group G.

Barratt and Garnjobst (1949) showed that gene pe^{m} (peach-microconidial) is independent of groups A through F but is apparently allelic with pe (peach), which is a marker in Lindegren's (1939) linkage group II. Thus linkage group G is Lindegren's group II. The gene pe has a centromere distance of 11.3 based on 278 asci (Lindegren, 1939) and pe^{m} a distance of 14.4 based on 392 asci (Barratt and Garnjobst, 1949). A linkage test with aro^{C120} would therefore be feasible.

Biochemistry

Mutant C120 grows maximally when supplied with the four aromatic compounds phenylalanine, tyrosine, tryptophane, and p-aminobenzoic acid. The results from tests of the various combinations of these are given in Table 6. Neither shikimic acid (Davis, 1950) nor (-)-quinic acid (Gordon, Haskins, and H. K. Mitchell, 1950) is active. Either arginine or lysine alone supports trace growth, and they are stimulatory in some combinations with other compounds (Table 6).

It may be noted that, of the four aromatic compounds, the only obviously stimulatory combination of two is phenylalanine plus tyrosine. The only combination of three which stimulates growth beyond this level is phenylalanine plus tyrosine plus tryptophane.

For the limited growth of C120 on phenylalanine plus tyrosine, the optimal mass ratio of l-tyrosine to dl-phenylalanine is ca. 2; but almost equal growth is obtained over the range 0.5 to ca. 3. Maximal growth cannot be obtained by adding large amounts of these two amino acids. At a total concentration of 0.05 mg./ml. with a mass ratio of 1.0, they support about one-third maximal growth. Inhibition is observed at higher concentrations.

Growth curves for the four aromatic compounds were determined individually when the concentrations of the remaining three were held constant at about optimal levels. These showed that C120 grows maximally with dl-phenylalanine at 0.025 mg./ml., l-tyrosine at 0.05 mg./ml., l-tryptophane at 0.025 mg./ml., and p-aminobenzoic acid at 1γ/ml.

Neither shikimic acid nor (-)-quinic acid will fulfill either the tyrosine requirement or the phenylalanine requirement. The tryptophane

Table 6. Growth of C120-A for 88 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	0.0
pab	0.0
ty	0.0
tr	0.0
ph	0.0
pab + ty	0.0
pab + tr	0.0
pab + ph	0.0
ty + tr	0.0
tr + ph	trace
ty + ph	2.8
pab + ty + tr	0.0
pab + ty + ph	2.1
pab + tr + ph	trace
ty + tr + ph	9.2
pab + ty + tr + ph	40.1
ty + tr + ph + a	15.8
ty + tr + ph + l	15.0
ty + tr + ph + a + l	14.1
a	trace
l	trace
a + l	trace

Key to symbols: a = l-arginine monohydrochloride at 0.05 mg./ml.; l = dl-lysine monohydrochloride at 0.1 mg./ml.; pab = p-aminobenzoic acid at 0.5 γ/ml.; ph = dl-phenylalanine at 0.1 mg./ml.; tr = l-tryptophane at 0.05 mg./ml.; ty = l-tyrosine at 0.05 mg./ml.

requirement was replaceable by anthranilic acid but not by nicotinamide, aniline, kynurenine, or indole in the one test made. In view of previous work (see review by H. K. Mitchell, 1951), these results should be checked. The requirement for p-aminobenzoic acid was replaceable by p-nitrobenzoic acid at 0.5 γ/ml. and apparently was partially fulfilled by dl-methionine at 0.1 mg./ml. Benzoic acid, p-hydroxybenzoic acid, aniline, o-phthalic acid, or nicotinic acid at 0.5 γ/ml. and shikimic acid or (-)-quinic acid at ca. 0.05 mg./ml. will not substitute for p-aminobenzoic acid.

Mutant C120 will grow poorly on known mixture A and no better on V A M-4 V-4, which contains the four aromatic compounds required. The two richest chemically undefined mixtures of those tested, are enzymatically hydrolyzed casein and liver extract. On the basis of paper chromatography, neither of these contains a compound which alone will suffice for the growth of C120, and which, of course, is stable in the solvents used.

C. Mutant Alpha-51 (C121 and C122)

Genetics

Four asci which had more than two mutant spore-pairs were obtained from the outcross of alpha-51-A to wild-type 5297-a. Mutant alpha-51 is therefore mutant for at least two genes. Growth tests on the mutant reisolates from these asci showed that they fell into three clear-cut classes: (1) those that needed only arginine, (2) those that needed only cytidine (or uridine), and (3) those that needed both. An arginineless (arg) reisolate has been given the occurrence designation C122, a pyrimidineless (pyr) one the designation C121. Of the four classifiable asci, two segregated in the second division for arg^{C122} and none segregated in the second division for pyr^{C121}.

Biochemistry

The original mutant alpha-51 will not grow on any of the known mixtures used, including V A M-4 V-4, which contains arginine and cytidine. Neurospora autolysate is the richest chemically undefined mixture of those tried.

The component mutant C121 will grow maximally on either cytidine sulfate or uridine at a concentration of 0.1 mg./ml. or higher. A combination of these two compounds in equal mass concentrations is not more active than either one alone. Mutant C121 will not utilize cytosine even at a concentration of 0.25 mg./ml., but uracil at that concentration supports ca. 10% maximal growth (cf. H. K. Mitchell and Houlahan, 1947).

The component mutant C122 will grow maximally on l-arginine monohydrochloride at a concentration of 0.175 mg./ml. Acid-hydrolyzed casein

is a rather poor source for the growth of C122. No inhibition is ascribable to dl-lysine, however. In combination with l-arginine, dl-lysine is stimulatory, as indicated by Table 7. When dl-lysine is tested alone, though, it is inactive.

Table 7. Growth of C122-a for 96 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	0.0
a	23.3
a + l (0.01 mg./ml.)	29.2
a + l (0.035 mg./ml.)	30.2
a + l (0.1 mg./ml.)	33.8
a + l (0.2 mg./ml.)	36.4
dl-ornithine monohydrochloride (0.1 mg./ml.)	15.2
dl-citrulline (0.1 mg./ml.)	60.0

Key to symbols: l = dl-lysine monohydrochloride; a = l-arginine monohydrochloride at 0.05 mg./ml. This arginine concentration allows growth limited to ca. 40% maximal.

The utilization of ornithine or citrulline, also shown in Table 7, indicates that the block in C122 is prior to ornithine in the metabolic path proposed by Srb (1947) for arginine biosynthesis in Neurospora.

D. Mutant Delta-19 (C123)

Genetics

The dissection of asci from outcrosses of delta-19 to wild type showed that delta-19 is probably a single-gene mutant. The new occurrence designation C123 was given to the original stock. The mutant gene will therefore be referred to as $\underline{asg}^{C123}$ (asparagineless).

Surber (1951) found a mutant (1A7) which would utilize l-asparagine. She dissected asci from a cross of 1A7 and C123 and found no wild-type spore-pairs.

This work was extended for this report. From the cross 1A7-a x C123-A random ascospores were plated and classified on minimal agar. From an estimated total number of 10^5 spores, of which ca. 83% had germinated, only two wild types were found. Upon outcrossing to wild-type stocks, neither of these yielded mutant progeny; hence they were not pseudo-wild types (M. B. Mitchell, Pittenger, and H. K. Mitchell, 1952). One explanation of their origin would be through crossing-over between pseudoallelic $\underline{asg}^{C123}$ and $\underline{asg}^{1A7}$. In any case, it may be concluded that $\underline{asg}^{C123}$ and $\underline{asg}^{1A7}$ are at least closely linked. They will be considered as alleles in this investigation.

Another one of the "unknowns" (67603), on which research is reported later in this thesis, requires asparagine for nonadaptive growth. The crosses 1A7-a x 67603-A and 67603-A x C123-R-a gave no wild-type progeny among large numbers of random ascospores, as classified on plates of minimal agar.

It is assumed then that $\underline{asg}^{1A7}$, $\underline{asg}^{C123}$, and $\underline{asg}^{67603}$ are allelic.

The available data from the dissection of asci are combined in Table 8.

Table 8. Outcrosses of C123 and 67603 to wild-type strains

Cross	Percentage of Dissected Asci Classifiable	Number of Classifiable Asci	Number of Asci Seg. II for asg.
67603A and WP1347-2-a (H.K. Mitchell,1951)	100	12	10
5297-a x 67603-A	36	27	22
5297-a x C123-A	62	18	10
15300-R3-a(alb_2) x C123-A	58	7	5

Protoperithecial parent is listed before the crossing symbol "x."

These data give a centromere distance of 37 for asg.

Crosses were made to several marker genes in order to locate asg (Table 9).

The placement of pab^{1633} in linkage group E was made by E. Z. Lederberg and corroborated by Emerson (1952). The data show that asg is on the pab^{1633}-containing arm of linkage group E. A more specific genetic placement is not possible from the data. In fact, apparently non-random genetic recombination with both of the more proximal markers (lys^{33933} and $isol\text{-}val^{16117}$) is surprising. These two markers have been listed on opposite sides of the centromere (Barratt and Garnjobst, 1949); and if asg does segregate at random with respect to the centromere as the data indicate, then one would not expect it to show linkage with a marker on the other side of the centromere.

Table 9. Progeny from crosses of C123 and 1A7 to marker genes

Cross	Marker Gene Its Linkage Group and Centromere Distance	Percentage Germination	Number of Germinated Spores Classified	Percentage Classified as Wild-Type	Classifiability
1A7-a x 10575-A	tryp; C; 15.2	94	634	24	good
1A7-a x 263-R1-A	pyr; D; 5.72	47	159	28	fair
C123-A x 33933-P1-a	lys; E; 4.8	58	405	11	fair
C123-A x 16117-a	isol-val; E; 8.9	86	1128	14.8	good
1A7-a x 1633-A	pab; E; 29.6	74	619	13	good
1633-A x 1A7-a	pab; E; 29.6	56	254	11	good
1A7-a x E15172-16014-A	sfo; F; less than 1	74	649	27	fair

Protoperithecial parent is listed first. Classifications were made on agar plates.

It is interesting to note that stocks with the gene $\underline{asg}^{C123}$ have been observed to revert to wild type several times, whereas no such reversions have been observed in cultures with $\underline{asg}^{1A7}$ or with $\underline{asg}^{67603}$. One of these reverted cultures was outcrossed to wild type and no mutants were recovered. It therefore seems likely that the genetic change was not suppression.

Biochemistry

Mutant C123 grows maximally when supplied with l-asparagine at a concentration of 0.2 mg./ml. or higher.

Tests of l-aspartic acid, l-glutamic acid, and l-glutamine, alone and in all combinations thereof, were inactive for this mutant. Other compounds which are inactive, as determined by individual tests, include all the components of known mixture V A M-6 Vr-l Ex-l and thymine, citrulline, ornithine, norvaline, norleucine, creatine, adenosine, guanine, α-amino-n-butyric acid, β-alanine, cysteine, glutathione, and uracil.

Of the chemically undefined mixtures tested, Neurospora autolysate and liver extract are the richest. Chromatopiles of both of these crude mixtures were developed in a solvent of five volumes n-propanol to one volume 0.6 N NH_4OH. Bioassays of these piles revealed peaks at Rf's 0.09 and 0.08 for Neurospora autolysate and liver extract, respectively. These peaks from the two piles were combined and further resolved on another chromatopile in a different solvent (five volumes n-propanol to one volume 0.6 N HCl). Bioassay of this pile showed a single peak at Rf 0.07. These Rf values are not very different from

those expected for asparagine in these systems. It is likely then that the activities of Neurospora autolysate and liver extract are ascribable to contained asparagine.

E. Mutant 35709 (C125 and 35709)

Genetics

H. K. Mitchell (1951) outcrossed this to wild type and four of the six complete asci tested had more than two mutant spore-pairs. The original 35709 is therefore mutant for at least two genes.

An ascus which had three mutant spore-pairs was chosen for further investigation. Table 10 reports growth data for these three mutant spore-pairs.

Table 10. Growth of mutant reisolates for 104 hours at 25° C.

Medium	Mycelial Mass (mgs.) D1	D2	D3
M	30.0	2.1	0.2
N	56.5	44.3	77.5
V	28.7	2.7	0.3
A	27.8	27.8	56.6
M-6	20.7	8.6	17.7
Vr-3	26.1	2.9	0.7
Ex-2	17.6	2.5	0.6
V A M-6 Vr-3 Ex-2	34.5	38.9	70.5

Reisolate D4 is wild type

In this same experiment visual estimates of growth were made after 70 hours. At that time neither D1 nor D2 had grown very much in any medium, whereas D3 had grown nearly maximally on N, A, and V A M-6 Vr-3 Ex-2. From this observation and from the data presented in Table 10,

it was inferred that D1 and D3 are the two single-gene mutant reisolates expected and that D2 is the double mutant.

Reisolate D1 is given the occurrence designation C125, and its mutant gene is assigned the tentative symbol $\underline{sl}^{C125}$ (slow-grower), since, as far as is known at present, C125 is a largely indispensable slow-growing mutant.

Reisolate D3 will grow maximally on homoserine (Teas, 1952). This reisolate retains the occurrence designation 35709, and its mutant gene will be referred to as $\underline{hmsr}^{35709}$.

In its utilization of homoserine in place of threonine plus methionine for maximal growth, mutant 35709 is similar to mutant 51504, which has been investigated rather extensively (Teas, 1946; Teas, 1947; Teas, Horowitz, and Fling, 1948; Fling and Horowitz, 1951). In its better utilization of threonine or isoleucine for growth, 35709 differs from 51504.

Teas has found that $\underline{hmsr}^{51504}$ is in linkage group A on the arm which does not contain the mating-type locus. He also showed that its centromere distance is 26 and that it gives very close linkage with either $\underline{alb}_1$ or $\underline{alb}_2$.

Random ascospores from a cross between 35709-D3-A and 51504-a were classified on a minimal plate. The cross was rather infertile and only a relatively small number of mature ascospores were produced. No wild types were found among ca. 10^2 germinated ascospores.

Table 11 gives the available data for the calculation of centromere distance for $\underline{hmsr}^{35709}$.

Table 11. Outcrosses of 35709-D3-A to nutritionally wild types

Cross	Percentage of Dissected Asci Classifiable	No. of Classifiable Asci	No. of Asci Seg. II for hmsr
5297-a x 35709-D3-A	50	13	12
15300-R3-a (*alb*$_2$) x 35709-D3-A	79	11	4

Protoperithecial parent is listed first.

These data give a centromere distance for *hmsr*^{35709} of 33, which is reasonably close to the value obtained by Teas for *hmsr*^{51504}. A classification for sex of spore-pairs from asci from the cross 5297-a (wild-type) x 35709-D3-A gave a recombination value of 48% between *hmsr*^{35709} and sex. A classification of random spores from the cross 15300-R3-a (*alb*$_2$) x 35709-D3-A, however, revealed close linkage between *alb*$_2^{15300}$ and *hmsr*^{35709}.

The conclusion may be drawn then that *hmsr*^{35709} is either allelic with or close to *hmsr*^{51504}.

If *hmsr*^{35709} is not allelic with *hmsr*^{51504} then it might be expected that mixed conidia of 35709 and 51504 would grow faster on minimal medium, through heterocaryotic or other syntrophy, than conidia of either one alone. With the cooperation and help of Dr. Teas, experiments along this line were performed with 51504-a and 12 reisolates of 35709 of mating-type a. Experiments were run on minimal medium and on minimal supplemented with dl-homoserine at a "starter" concentration of 1γ/ml. both in flasks with liquid media and in growth tubes with solid agar media. In none of the 12 possible combinations was growth of the mixed inocula reproducibly faster or greater than growth of both single

inoculum controls.

Biochemistry

The component mutant C125 has not been investigated further.

The component mutant 35709, as mentioned above, grows maximally on homoserine or on threonine plus methionine (Teas, 1952). Either threonine or isoleucine will support fair growth, and methionine is almost as active as either of these two. At low concentrations α-amino-n-butyric acid is as active as threonine, but at higher concentrations it is inhibitory (Teas, 1952). Succinic acid shows slight activity.

Mutant 35709 will grow slowly and adaptively on minimal medium. Leucine will inhibit this adaptive growth.

F. Mutant 36111

Genetics

Table 12 gives the available data for the calculation of centromere distance for pab^{36111}. These asci gave no indication that 36111 is not a single-gene mutant.

Table 12. Outcrosses of 36111 to wild types

Cross	Percentage of Dissected Asci Classifiable	No. of Classifiable Asci	No. of Asci Seg. II for pab^{36111}
36111-R1-A and WP1347-2-a (Mitchell, 1951)	90	9	8
5297-a x 36111-D8-A	80	28	18

C. D. = 35

A cross was made to the marker pab^{1633}, which has a centromere distance of 29.6 (Houlahan, Beadle, and Calhoun, 1949) and which is located in linkage group E (Emerson, 1952). Random ascospores from this cross were classified on a plate of minimal agar. The percentage germination was 47. Among 765 germinated ascospores classified there were no wild types.

Hence pab^{36111} is likely allelic with pab^{1633}.

Biochemistry

Mitchell (1951) demonstrated a utilization by 36111 of p-aminobenzoic acid.

The growth on p-aminobenzoic acid is maximal. Good adaptive growth can be obtained on p-nitrobenzoic acid; but p-hydroxybenzoic acid, methionine, arginine, lysine, adenine, and choline were inactive at the levels tested.

G. Mutant 37812

Genetics

Of 13 classifiable asci obtained from an outcross of 37812 to a wild-type strain, none showed more than two mutant spore-pairs (H. K. Mitchell, 1951). It is tentatively assumed then that 37812 is a single-gene mutant. Only one of these 13 asci segregated in the second meiotic division for the mutant gene, which is designated ace^{37812} (aceticless). This gives a centromere distance of four.

Biochemistry

Mutant 37812 grows poorly or not at all in flasks with any of the liquid media commonly used in this work. It does, however, grow well on agar slants supplemented with enzymatically hydrolyzed casein (Nutritional Biochemicals Corp.); and it will grow maximally on such agar slants when they are supplemented simply with acetic acid.

Compounds which are inactive for the growth of 37812 include all the amino acids present in acid-hydrolyzed casein, asparagine, glutamine, homoserine, succinic acid, sodium carbonate, sodium bicarbonate, formic acid, formaldehyde, methanol, acetaldehyde, ethanol, propionic acid, propionaldehyde, acetone, n-propanol, and iso-propanol. Acid-hydrolyzed casein is also inactive.

If enzymatically hydrolyzed casein is distilled at pH 2, the activity is found in the distillate. If this active fraction is redistilled at pH 12, the activity is in the residue. Such an active residue was derived with aniline and with p-toluidine. The two derivatives were recrystallized from aqueous alcohol and their melting-points were determined. (See Table 13).

Table 13. Melting points of derivatives

Derivative	Observed (uncorrected) (°C.)	Literature Values for These Derivatives of Acetic Acid (°C.) Source (1)	Source (2)
anilide	112.8	113.4	114
p-toluidide	143.5	153	147

Sources: (1) Lange's Handbook of Chemistry, Sixth Edition (1946); (2) Shriner, R. L., and R. C. Fuson, "The Systematic Identification of Organic Compounds" (1948).

It is suggested that Nutritional Biochemicals Corp. buffers their enzymatically hydrolyzed casein (at pH 4) with acetate.

H. Mutant 44303T (C124 and 44303T)

Genetics

Asci were dissected from an outcross of 44303T-A to wild-type strain WP1347-2-a. A classification of these on a plate of minimal medium indicated that some had more than two mutant spore-pairs. It has been assumed then that the original 44303T-A is a double mutant. A determination of the growth requirements of the mutant reisolates has led to the following classification of these asci (Table 14).

Table 14. Genotypes of progeny from cross between 44303T-A and WP1347-2-a (wild-type)

	Genotypes of Progeny in Ascus			
Ascus No.	Pair A	Pair B	Pair C	Pair D
3	meth+aspt	meth+aspt	+methaspt	+methaspt
4	meth aspt	meth aspt	+meth+aspt	+meth+aspt
6	+meth+aspt	+meth+aspt	meth aspt	meth aspt
10	+meth+aspt	+meth+aspt	meth aspt	lost
11	meth aspt	meth aspt	+meth+aspt	+meth+aspt
12	X	+methaspt	meth+aspt	meth+aspt
13	meth aspt	meth aspt	+meth+aspt	+meth+aspt
14	meth+aspt	meth+aspt	+methaspt	+methaspt
15	+meth+aspt	+meth+aspt	meth aspt	meth aspt

C.D. of meth^{C124} = 0; C.D. of aspt^{44303T} = 0

Key to symbols: meth = methionineless; aspt = asparticless; X = failed to germinate.

Reisolate 44303T-14A-a (methionineless) has been given the new occurrence designation C124. Reisolate 44303T-14D-A (asparticless) retains the occurrence designation 44303T.

Gershowitz (1952) has reported further data on the centromere distance of $meth^{C124}$. All the data are compiled in Table 15.

Table 15. Available data for calculation of centromere distance for $meth^{C124}$

Cross	Percentage of Dissected Asci Classifiable	No. of Classifiable Asci	No. of Asci Seg. II for $meth^{C124}$
44303T-A & WP1347-2-a	60	9	0
Outcross to wild type (Gershowitz, 1952)	40	31	0

C.D. = 0

Biochemistry

The component mutant C124 (meth) will grow maximally when supplied with dl-methionine at a concentration of 0.05 mg./ml. or higher. The compounds l-cystine, dl-serine, cysteic acid, l-cysteine, hydrochloride, taurine, dl-homocystine, and homocysteine thiolactone hydrochloride are inactive when tested alone. The growth obtained on known mixture M-4, which contains dl-methionine and adenine sulfate, is no better than on dl-methionine alone.

The component mutant 44303T-14D (aspt) grows adaptively on minimal medium at either 25° C. or 36° C. Since this adaptive growth is more rapid at 25° C., growth tests are clearest when performed at 36° C. Neurospora autolysate is a good complex source for growth. Acid-hydrolyzed

casein is also a good source, and its activity can be equaled by synthetic mixtures of amino acids. Of the amino acids tested individually, l-aspartic acid is the most active. Its homolog l-glutamic acid is nearly as active, but a mixture of equal masses of these two amino acids in one test was not more active than l-aspartic acid alone. Many other amino acids are also active. However, dl-alanine is inhibitory. Some of the growth data are given in Table 16.

Table 16. Growth of 44303T-14D-A at 36° C.

Medium	Mycelial Mass (mgs.)
Experiment #1	
M	7.5
N	54.1
A	37.0
dl-homoserine (0.1 mg./ml.)	19.6
dl-isoleucine (0.1 mg./ml.) + dl-valine (0.2 mg./ml.)	14.6
g (0.05 mg./ml.) + al (0.1 mg./ml.) + aspt (0.05 mg./ml.)	24.8
Experiment #2	
M	7.6
N	55.7
A	31.2
g (0.025 mg./ml.)	13.6
g (0.10 mg./ml.)	18.9
g (0.25 mg./ml.)	19.2
g (0.60 mg./ml.)	20.5
g (1.0 mgl/ml.)	19.9
g (1.8 mg./ml.)	15.0
aspt (0.025 mg./ml.)	15.5
aspt (0.25 mg./ml.)	28.8
aspt (1.8 mg./ml.)	16.5
al (0.050 mg./ml.)	2.1
al (0.50 mg./ml.)	2.1
al (3.4 mg./ml.)	1.7
l-leucine (0.81 mg./ml.)	18.3

Table 16. (Continued)

The growth periods are 101 hours in experiment #1 and 96 hours in experiment #2. Key to symbols: al = dl-alanine; aspt = l-aspartic acid; g = l-glutamic acid.

It is apparent that the designation asparticless for the mutant gene is a rather arbitrary one. It has been used simply for its convenience.

I. Mutant 45208T

Genetics

Houlahan, Beadle, and Calhoun (1949) reported 18 asci, each of which had at least three spore-pairs represented, from an outcross of this "unknown" to wild type. In this investigation, seven complete asci were obtained. None of these 25 showed more than two mutant spore-pairs. It is therefore likely that 45208T is a single-gene mutant or is mutant for two or more linked genes.

The available data for calculation of centromere distance are presented in Table 17.

Table 17. Outcrosses of 45208T to wild-type strains

Cross	Percentage of Dissected Asci Classifiable	No. of Classifiable Asci	No. of Asci Seg. II for leu 45208T
45208T-a x Abbott 4-A (wild-type) (Houlahan, Beadle, and Calhoun, 1949)	60	18	11
45208T-C1-a x WP14004-A (wild-type)	53	8	4

C. D. = 29

Biochemistry

This mutant grows adaptively on minimal medium at 25° C. When supplied with l-leucine at 25° C., it grows nonadaptively. At 36° C. it does not grow on minimal medium and grows only slowly on a medium supplemented with l-leucine. Liver extract (Lilly), which supports nonadaptive growth at 25° C., supports no growth at 36° C. The amino acids dl-isoleucine and dl-phenylalanine are inhibitory. Tables 18 and 19 give some of the pertinent data.

Table 18. Growth of 45208T-9B-A for 136 hours

Medium	Mycelial Mass (mgs.) 25° C.	36° C.
M	31.8	--
Lv	70.8	0.0
l-leucine (0.05 mg./ml.)	68.3	--
dl-isoleucine (0.1 mg./ml.)	1.3	--
dl-phenylalanine (0.1 mg./ml.)	3.5	--

Table 19. Growth of 45208T-9B-A for 116 hours

Medium	Mycelial Mass (mgs.) 25° C.	36° C.
M	25.3	0.0
l-leucine (0.05 mg./ml.)	49.2	7.3
l-leucine (0.15 mg./ml.)	57.2	--
l-leucine (0.2 mg./ml.)	--	11.3
l-leucine(0.1 mg./ml.)+dl-isoleucine(0.05 mg./ml.)	50.1	--
l-leucine(0.05 mg./ml.)+dl-isoleucine(0.1 mg./ml.)	38.8	trace
dl-isoleucine (0.15 mg./ml.)	trace	--
l-leucine (0.1 mg./ml.) + dl-phenylalanine (0.05 mg./ml.)	57.3	--
l-leucine (0.05 mg./ml.) + dl-phenylalanine (0.1 mg./ml.)	31.8	0.0
dl-phenylalanine (0.15 mg./ml.)	0.2	--

J. Mutant 46005

Genetics

The genetic data for this "unknown" are meager. Mitchell (1951) reported no evidence that this is not a single-gene mutant. Of nine classifiable asci, four segregated in the second division.

Biochemistry

This mutant grows well when supplied with succinic acid, some of the other members of the citric acid cycle, l-asparagine, or l-glutamine. It will grow adaptively on sodium acetate.

Acid-hydrolyzed casein supports good growth, but no one of its component amino acids is very active. The most active are l-aspartic acid and l-glutamic acid. Some of the growth information is given in Tables 20 and 21.

Table 20. Growth of 46005-a for 124 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	trace
succinic acid (0.0025 mg./ml.)	0.7
succinic acid (0.015 mg./ml.)	10.4
succinic acid (0.06 mg./ml.)	40.5
succinic acid (0.2 mg./ml.)	45.2
succinic acid (0.3 mg./ml.)	40.4
l-asparagine (ca. 0.08 mg./ml.)	37.7
l-glutamine (ca. 0.08 mg./ml.)	19.5
acid-hydrolyzed casein (0.5 mg./ml.)	34.2
l-aspartic acid (0.05 mg./ml.)	0.8
l-glutamic acid (0.05 mg./ml.)	0.9

The l-glutamine was added in crystalline form after autoclaving.

Table 21. Growth of 46005-a for 102 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	trace
succinic acid (0.1 mg./ml.)	32.6
fumaric acid (0.05 mg./ml.)	9.6
dl-malic acid (0.1 mg./ml.)	27.9
sodium pyruvate (0.075 mg./ml.)	trace
sodium acetate (0.075 mg./ml.)	2.0
citric acid (0.05 mg./ml.)	trace
cis-aconitic acid (ca. 0.1 mg./ml.)	trace
dl-isocitric acid (ca. 0.1 mg./ml.)	trace
oxalosuccinic acid (ca. 0.1 mg./ml.)	3.8
α-ketoglutaric acid (0.05 mg./ml.)	8.9

The dl-isocitric acid, oxalosuccinic acid, and cis-aconitic acid were added in crystalline form after autoclaving.

K. Mutant 46403

Genetics

Houlahan, Beadle, and Calhoun (1949) reported no evidence from 19 asci each of which had at least three spore-pairs represented, that mutant 46403 is not a single-gene mutant. They placed the gene $\underline{suc}^{46403}$ in linkage group A on the arm which does not contain the mating-type locus. The centromere distance of $\underline{suc}^{46403}$, based on these 19 asci, is 16.

Biochemistry

This mutant is very similar to 46005 in its growth behavior. It grows maximally on α-ketoglutaric acid, succinic acid, fumaric acid, dl-malic acid, or acid-hydrolyzed casein. Good nonadaptive growth is supported by either l-glutamine or l-asparagine. Sodium acetate supports good adaptive growth, and fair adaptive growth is obtained with l-aspartic acid plus l-glutamic acid.

Many compounds other than those listed in the tables below were tested individually. These all supported no growth or only trace growth. Those tested include all the other amino acids present in acid-hydrolyzed casein, β-alanine, dl-ornithine monohydrochloride, lactic acid, sodium pyruvate, citric acid, dl-isocitric acid, n-propionic acid, n-butyric acid, n-valeric acid, n-caproic acid, oxalic acid, malonic acid, glutaric acid, adipic acid, maleic acid, and trans-aconitic acid.

Some of the data are presented in Table 22.

Table 22. Growth of 46403-a at 25° C.

Medium	Mycelial Mass (mgs.)
Experiment #1	
M	0.0
succinic acid (0.0025 mg./ml.)	2.0
succinic acid (0.015 mg./ml.)	12.8
succinic acid (0.06 mg./ml.)	63.6
succinic acid (0.2 mg./ml.)	76.6
succinic acid (0.3 mg./ml.)	73.4
α-ketoglutaric acid (0.05 mg./ml.)	31.6
fumaric acid (0.05 mg./ml.)	35.4
dl-malic acid (0.1 mg./ml.)	35.9
sodium acetate (0.075 mg./ml.)	23.2
l-aspartic acid (0.05 mg./ml.)	0.1
l-glutamic acid (0.05 mg./ml.)	2.0
l-aspartic acid (0.05 mg./ml.) + l-glutamic acid (0.05 mg./ml.)	2.5
l-tyrosine (0.05 mg./ml.)	0.5
l-asparagine (ca. 0.08 mg./ml.)	5.4
l-glutamine (ca. 0.08 mg./ml.)	27.4
Experiment #2	
M	0.0
succinic acid (0.1 mg./ml.)	56.5
oxalosuccinic acid (ca. 0.05 mg./ml.)	19.8
cis-aconitic acid (ca. 0.05 mg./ml.)	1.4
l-tyrosine (ca. 0.05 mg./ml.)	0.5

Table 22 (Continued)

Medium	Mycelial Mass (mgs.)
Experiment #3	
M	0.3
succinic acid (0.1 mg./ml.)	85.8
A	85.2
l-aspartic acid (0.05 mg./ml.) + l-glutamic acid (0.05 mg./ml.)	28.9
l-aspartic acid (0.05 mg./ml.) + l-glutamic acid (0.05 mg./ml.) + l-tyrosine (0.05 mg./ml.)	51.2

The growth periods were for 124 hours in experiment #1, for 102 hours in experiment #2, and for 136 hours in experiment #3. The l-asparagine and l-glutamine in experiment #1 and the oxalosuccinic acid, cis-aconitic acid, and l-tyrosine in experiment #2 were added to the flasks in crystalline form after autoclaving.

Since oxalosuccinic acid is thermolabile and decarboxylates to give active α-ketoglutaric acid, it cannot be concluded from the above data that oxalosuccinic acid itself is active.

L. Mutant 47903

Genetics

H. K. Mitchell (1951) obtained data which indicated that 47903 is a single-gene mutant or is mutant for two or more linked genes. Of 12 classifiable asci, two segregated in the second division for the mutant gene, which is designated *ser*^{47903} (serineless).

Intercrosses between 47903 and three mutant strains, which are somewhat similar with respect to the compounds they utilize for growth, indicate non-allelism (Table 23).

Which parent is the protoperithecial one in these crosses cannot be definitely stated. The strains indicated as protoperithecial parents are simply those which were given time to form protoperithecia before the crosses were actually made. Since the germination percentages obtained in reciprocal crosses may be different, a comparison of the data from these crosses may afford an indication as to the direction of any bias stemming from differential germination of genotypes.

Table 23 indicates that *ser*^{47903} segregates at random with respect to *for*^{C24}, *mac*^{65108}, and *ser*^{65004}.

The mutant strain C24 was shown to utilize formic acid by Harrold and Fling (1952). Mutant 65004 will utilize formic acid adaptively. Mutant 65108 will not utilize formic acid but will grow maximally on methionine plus adenine plus cystine. Mutants 65004 and 65108 are discussed later in this thesis.

Table 23. Progeny from crosses of 47903 to C24, 65108, and 65004

Cross and Cross No.	Percentage Germination	No. of Germinated Spores Classified	Percentage Classified as Wild Type	Classifiability
(1) 47903-D2-a x C24-A(for)	94	115	29	fair
(2) Reciprocal of (1)	91	389	28	poor
(3) 47903-D2-a x 65108-III 1(8)-A(mac)	46	148	20	fair
(4) Reciprocal of (3)	95	310	24	good
(5) 47903-D2-a x 65004-A(ser)	90	504	26	fair
(6) Reciprocal of (5)	81	56	21	fair

Protoperithecial parent is listed first.

Biochemistry

Mutant 47903 will grow well on serine, but it will grow only in trace amounts on either acid-hydrolyzed or enzymatically hydrolyzed casein. "Bacto-Peptone" is the best complex source of those which were tested. A chromatopile of this crude source revealed a peak of high activity for 47903 at Rf 0.39, which is ca. the Rf of serine on a chromatopile in the solvent used (five volumes n-propanol to two volumes 0.4 N NH_4OH). A smaller peak, which supported adaptive growth, was found at Rf 0.61.

Mutant 47903 will also grow moderately well on adenine plus methionine plus tryptophane plus lysine. The growth data for all possible combinations of these compounds are presented in Table 24.

These data show that the only combination of two which is very noticeably stimulatory is adenine plus methionine. They also show that the only combination of three which stimulates beyond this level is adenine plus methionine plus tryptophane.

Choline alone is not active at the concentration tested; but if it is supplied in addition to the four compounds considered in Table 24, somewhat better growth is obtained (Table 25). Riboflavin, dl-phenylalanine, and l-tyrosine inhibit the growth of 47903 as supported by adenine plus methionine plus tryptophane plus lysine (Table 25).

Mutant 47903 will utilize formic acid or formaldehyde (Table 26). These compounds were usually added to the flasks after autoclaving. They inhibited the growth of wild-type strain 5297-a when they were added and tested in this way. Formic acid at 0.55 mg./ml. gave 84% inhibition, and formaldehyde at 0.075 mg./ml. gave 96% inhibition.

Table 24. Growth of 47903-D5-a for 101 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	trace
"Bacto-Peptone" (Difco) (2.8 mg./ml.)	48.0
a	trace
m	trace
t	trace
l	trace
a + m	6.1
a + t	trace
a + l	trace
m + t	trace
m + l	trace
t + l	0.5
a + m + t	14.0
a + m + l	4.4
a + t + l	1.7
m + t + l	trace
a + m + t + l	22.2

Key to symbols: a = adenine sulfate at 0.04 mg./ml.; m = dl-methionine at 0.1 mg./ml.; t = l-tryptophane at 0.05 mg./ml.; l = dl-lysine monohydrochloride at 0.1 mg./ml.

Table 25. Growth of 47903-D5-a at 25° C.

Medium	Mycelial Mass (mgs.) Exp. #1	Exp. #2
M	trace	trace
"Bacto-Peptone" (Difco) (ca. 2 mg./ml.)	43.8	43.7
a + m + t + l	22.2	8.6
a + m + t + l + choline chloride (2γ/ml.)	25.7	22.8
choline chloride (2γ/ml.)	--	trace
a + m + t + l + riboflavin (0.5γ/ml.)	3.4	3.5
a + m + t + l + dl-phenylalanine (0.1 mg./ml.) + l-tyrosine (0.05 mg./ml.) + p-aminobenzoic acid (0.5γ/ml.)	3.3	--
a + m + t + l + p-aminobenzoic acid (0.5γ/ml.)	--	7.1
a + m + t + l + dl-phenylalanine (0.1 mg./ml.)	--	1.1
a + m + t + l + l-tyrosine (0.05 mg./ml.)	--	2.7

The growth periods are for 101 hours in experiment #1 and for 98 hours in experiment #2. See Table 24 for symbols.

Table 26. Growth of 47903-D5-a for 98 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	trace
dl-serine (0.075 mg./ml.)	25.3
formic acid (0.025 mg./ml.)	2.9
formic acid (0.05 mg./ml.)	9.3
formic acid* (0.05 mg./ml.)	5.0
formic acid (0.1 mg./ml.)	15.4
formic acid (0.2 mg./ml.)	11.7
formic acid (0.375 mg./ml.)	2.3
formaldehyde (0.015 mg./ml.)	2.9
formaldehyde (0.04 mg./ml.)	trace
formaldehyde* (0.04 mg./ml.)	5.2
formaldehyde (0.065 mg./ml.)	0.0
formaldehyde (0.125 mg./ml.)	0.0

Formic acid and formaldehyde were dispensed after autoclaving in all cases except those marked with an asterisk (*).

M. Mutant 65004

Genetics

The available data for the calculation of centromere distance are compiled in Table 27.

Table 27. Outcrosses of 65004 to nutritionally wild-type strains

Cross	Percentage of Dissected Asci Classifiable	No. of Classifiable Asci	No. of Asci Seg. II for for^{65004}
65004-A and WP1347-2-a	53	8	5
5297-a x 65004-A	33	5	2
15300-R3-a (alb_2) x 65004-A	70	7	5

C. D. = 30

Of the 20 classifiable asci listed, 13 were complete. None of these showed more than two mutant spore-pairs. It is therefore likely that 65004 is not mutant for two unlinked genes.

The seven classifiable asci in the cross to the marker alb_2^{15300} contained 10 parental and 18 recombinant spore-pairs. Three of these seven asci had all four of their spore-pairs recombinant. In addition to this, 70 random spores from this cross were transferred to tubes for classification. Of the 58 of these which were classifiable for both ser^{65004} and alb_2^{15300}, 30 were recombinant. These data then indicate that ser^{65004} segregates independently of alb_2^{15300}, which is a marker in linkage group A.

It is very likely that ser^{65004} also segregates independently of ser^{47903}, a gene whose phenotype is similar. (Refer to the section on "unknown" mutant 47903.)

The data from two other intercrosses are presented in Table 28.

Table 28. Progeny from crosses of 65004 to C24 and 44601

Cross	Percentage Germination	No. of Germinated Spores Classified	Percentage Classified as Wild Type	Classifiability
65004-A x 44601-D2-a(ad-arg)	99	518	20	fair
65004-A x C24-a (for)	70	178	8.4	poor

Protoperithecial parent is listed first.

The gene ad-arg^{44601} (adenine-arginineless; see Introduction) is apparently near a centromere but its linkage group is not known (Houlahan, Beadle, and Calhoun, 1949). The gene for^{C24} is probably not in linkage groups A through E. (Refer to the section on "unknown" mutant alpha-10.) Especially, since the classifiability of the progeny from the cross to the marker for^{C24} was poor, the linkage suggested between this marker and ser^{65004} should be checked.

Biochemistry

In its utilization of serine or formic acid, mutant 65004 is similar to 47903, which has been considered earlier in this thesis. "Bacto-Peptone" (Difco) is the best complex source for 65004. This source was resolved on a chromatopile in a solvent of five volumes n-propanol to one volume 0.4 N NH_4OH. A bioassay of this pile showed a peak of activity at Rf 0.38, which corresponds with the Rf of serine in this system. A smaller peak was obtained at Rf 0.61. These results correspond almost exactly with those obtained with mutant 47903.

Although 65004 utilizes serine, neither enzymatically hydrolyzed nor acid-hydrolyzed casein will support more than trace growth.

The compounds which have been tested individually and found to be inactive include all of the components of V-1, A, M-6, Vr-3, and Ex-1, in addition to guanine hydrochloride and l-glutamine.

Some pertinent data are given in Table 29.

Table 29. Growth of 65004-II 15(1)-A for 116 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	0.0
"Bacto-Peptone" (Difco) (2 mg./ml.)	42.2
dl-serine (0.1 mg./ml.)	12.6
formic acid (0.1 mg./ml.)	0.8

The formic acid was added after autoclaving.

N. Mutant 65108T (C128T and 65108)

Genetics

Classifications, which were made on plates of minimal agar, of asci from outcrosses of "unknown" 65108 to two wild types (WP1400-4-A and 5256-A) indicated that 65108 was mutant for at least two genes. Some of these asci were transferred to tubes of complex medium N in order to establish reisolate cultures for further investigation. An unusually high proportion of the ascospores which were so transferred, however, died. Only two complete asci were obtained. Further growth tests showed that one of these had two and the other had three mutant spore-pairs. Table 30 gives some growth data for the reisolate cultures of the ascus with three mutant spore-pairs.

The simplest explanation of these data is that the parent 65108-a is a double mutant. The reisolate pair B (ascospores three and four) would then be this double mutant. Reisolate pair A (represented only by ascospore one) would be one component single-gene mutant, and reisolate pair D (ascospores seven and eight) would be the other. Pair C (ascospores five and six) is wild type.

A similar analysis was made of the other complete ascus, which had only two mutant spore-pairs. Each of these two spore-pairs was similar to the parental 65108-a in its growth behavior. This then corroborates the interpretation made above.

Further confirmation comes from data gained from outcrossing members of the pairs A, B, and D (of the ascus considered in Table 30) to nutritional wild-type strains. See Table 31.

Table 30. Growth of original 65108-a and of reisolates from the cross 5256-A (wild-type) x 65108-a at 25° C.

	Mycelial Mass (mgs.)							
	Parent	Ascospore No.						
Medium	65108-a	1	3	4	5	6	7	8
M	trace	42.8	0.0	0.0	84.3	79.6	trace	trace
m	6.6	45.8	trace	trace	--	--	36.1	37.9
a	trace	41.2	0.0	0.0	--	--	trace	trace
c	trace	39.8	0.0	trace	--	--	trace	trace
m + a	22.2	44.0	8.8	21.7	--	--	67.6	54.6
m + a + c	32.4	45.0	17.7	26.4	--	--	83.5	59.3
N	33.9	76.5	18.0	26.2	--	--	59.1	39.4
C	--	74.1	22.0	65.1	--	--	37.0	72.9

The growth period for the reisolates was 100 hours. The data for the original strain 65108-a are from a separate experiment, in which the growth period was 98 hours. The reisolates are from a single ascus. Ascospore #2 died. Key to symbols: m = dl-methionine at 0.1 mg./ml.; a = adenine sulfate at 0.04 mg./ml.; c = l-cystine at 0.05 mg./ml.

Table 31. Outcrosses to nutritionally wild-type strains of three reisolates from the cross 5256-A (wild-type) x 65108-a

Cross	Percentage Germination	No. of Germinated Spores Classified	Percentage Classified as Wild-type	Classifiability
WP1400-4-A x 65108-III 1(1)-a	86	110	59	poor
WP1400-4-A x 65108-III 1(3)-a	74	226	23	poor
15300-R3-a x 65108-III 1(8)-A	98	544	54	good

Reisolate III 1(1) represents pair A, III 1(3) represents pair B, and III 1(8) represents pair D. (See Table 30.) The classifications were made on plates of minimal agar.

Reisolate 65108-III 1(1)-a has been given the new occurrence designation C128T. Since the requirements of C128T for maximal growth at 25° C. have not been elucidated (see following section on biochemistry), its mutant gene carried the symbol $\underline{un}^{C128T}$ (unknown). No further genetic work has been done with C128T.

Reisolate 65108-III 1(8)-A retains the occurrence designation 65108. Its mutant gene has been assigned the symbol $\underline{mac}^{65108}$ (methionine-adenine-cystineless), since maximal nonadaptive growth is obtained on methionine plus adenine plus cystine.

This gene $\underline{mac}^{65108}$ has been located in the A linkage group about four genetic units from $\underline{alb}_2^{15300}$ (Table 32).

Table 32. The numbers of asci of different types from the cross 15300-R3-a (alb_2) x 65108-III 1(8)-A (*mac*).

Type of Ascus				
Type No.	Seg. of *mac*^{65108}	Seg. of alb_2^{15300}	Number of Recombinant Spore-Pairs	Number of Asci
1	I	I	0	9
2	I	I	4	0
3	I	II	2	0
4	II	I	2	0
5	II	II	0	4
6	II	II	2	1
7	II	II	4	0

% recombination = 4; C. D. of *mac*^{65108} = 18

The type numbers used here correspond to those used by Houlahan, Beadle, and Calhoun (1949).

A classification was made on a plate of minimal medium of random ascospores from the cross 65108-III 1(8)-A x 44601-D2-a (*ad-arg*). The percentage germination was 89, and the classifiability was good. Of the 282 germinated spores classified, 28% were classified as wild type. This would indicate random recombination between *mac*^{65108} and *ad-arg*^{44601}. The gene *mac*^{65108} has also been shown to segregate independently of *ser*^{47903}. (Refer to section on "unknown" 47903.)

Biochemistry

Some growth data are given for C128T in the above section on genetics (see Table 30). Neurospora autolysate will stimulate the growth

of C128T at 25° C., but a wild-type growth rate has not been obtained at this temperature. At 36° C., however, the growth behavior of C128T is wild type (Table 33).

Table 33. Growth of C128T-a for 96 hours

Medium	Mycelial Mass (mgs.) 25° C.	36° C.
M	9.2	64.5
N	51.3	76.8
acid-hydrolyzed casein (0.5 mg./ml.)	8.1	--
A	4.6	--
Ex-3	5.2	--
Vr-2	16.5	--
Vr-3	8.9	--
dl-lysine monohydrochloride(0.1 mg./ml.)	10.3	--

No further growth studies have been made with C128T. Its requirement(s) for optimal growth at 25° C. is chemically unknown.

Reisolate 65108-III 1(8)-A grows well on methionine plus adenine, but it will grow somewhat better if cystine is also supplied. The growth on methionine alone is adaptive. (Refer to growth data on ascospores seven and eight in Table 30.)

Growth studies have been performed with another reisolate, 65108-II 2(16), which, according to growth tests, possesses the gene $\underline{mac}^{65108}$ but not $\underline{un}^{C128T}$. It is then of the same genotype as 65108-III 1(8)-A. Neither formic acid nor formaldehyde is active in supporting the growth of 65108-II 2(6). Both of these compounds were dispensed to the test

flasks after autoclaving. Formic acid was tested at 0.025, 0.1 and 0.2 mg. per ml., and formaldehyde at 0.015, 0.075, and 0.175 mg. per ml.

The results from another growth experiment with 65108-II 2(6) are summarized in Table 34.

Table 34. Growth of 65108-II 2(6) for 100 hours at 25° C.

Medium	Mycelial Mass (mgs.)
M	0.3
N	41.6
choline chloride (0.025 mg./ml.)	trace
dl-serine (0.1 mg./ml.)	0.2
c (0.05 mg./ml.)	0.5
homocysteine thiolactone hydrochloride (ca. 0.1 mg./ml.)	0.3
a (0.04 mg./ml.) + c (0.05 mg./ml.)	0.6
a (0.04 mg./ml.) + homocysteine thiolactone hydrochloride (ca. 0.1 mg./ml.)	0.3
m (0.005 mg./ml.)	2.2
m (0.025 mg./ml.)	13.9
m (0.05 mg./ml.)	27.3
m (0.1 mg./ml.)	45.5
m (0.175 mg./ml.)	40.7
m (0.432 mg./ml.)	42.7
m (0.618 mg./ml.)	40.2
m (0.1 mg./ml.) + a (0.0025 mg./ml.)	47.7
m (0.1 mg./ml.) + a (0.015 mg./ml.)	55.6
m (0.1 mg./ml.) + a (0.04 mg./ml.)	64.8

Table 34 (Continued)

Medium	Mycelial Mass (mgs.)
m (0.1 mg./ml.) + a (0.05 mg./ml.)	55.4
m (0.1 mg./ml.) + a (0.2 mg./ml.)	64.2
m (0.1 mg./ml.) + adenosine (0.05 mg./ml.)	47.7
m (0.1 mg./ml.) + adenosine-3-phosphoric acid(0.05 mg./ml.)	53.8
m (0.1 mg./ml.) + guanine hydrochloride (0.04 mg./ml.)	4.4
m (0.1 mg./ml.) + guanosine (0.05 mg./ml.)	9.4
m (0.1 mg./ml.) + cystosine (0.04 mg./ml.)	43.9
m (0.1 mg./ml.) + cytidine sulfate (0.04 mg./ml.)	45.4
m (0.1 mg./ml.) + uracil (0.04 mg./ml.)	41.0
m (0.1 mg./ml.) + uridine (0.04 mg./ml.)	35.3
m (0.1 mg./ml.) + thymine (0.04 mg./ml.)	48.6
m (0.1 mg./ml.) + thymidine (0.04 mg./ml.)	31.3

The homocystine thiolactone hydrochloride was added in crystalline form after autoclaving. The symbols are defined as follows: a = adenine sulfate; c = l-cystine; m = dl-methionine.

These results indicate that maximal growth cannot be obtained simply by adding large amounts of dl-methionine. Homocysteine will not replace methionine; but it is likely that either adenosine or adenosine-3-phosphoric acid will fulfill the adenine requirement, at least partially. (The stimulation from either adenosine or adenosine-3-phosphoric acid was more obvious after a shorter growth period.) Both guanine and guanosine inhibit the adaptive growth supported by dl-methionine alone.

O. Mutant 67603T

Genetics

The dissection and classification of 39 asci showed that 67603T is probably a single-gene mutant. The gene $\underline{asg}^{67603T}$ is allelic with $\underline{asg}^{C123}$ and $\underline{asg}^{1A7}$. (Refer to the section on "unknown" delta-19 for data and further information.)

Biochemistry

Mutant 67603T grows nonadaptively when supplied with l-asparagine. On minimal medium at 25° C., it grows adaptively very well. At 36° C., however, this adaptive growth is less (Table 35).

Table 35. Growth of 67603T-A on minimal medium

Duration of Growth (hrs.)	Mycelial Mass (mgs.)		
	25° C.		36° C.
	1	2	
70	2.8	1.8	--
86	8.3	10.4	--
97	22.0	25.0	1.3
116	37.9	27.8	--
160	34.8	30.2	--

The tests at 25° C. were in duplicate, as shown.

A growth curve on l-asparagine showed that maximal nonadaptive growth is obtained with l-asparagine at 0.25 mg./ml.

The amino acids l-glutamine, l-glutamic acid, and l-aspartic acid are not stimulatory when they are tested alone or in any combination of

two. Other compounds which are inactive when tested alone include succinic acid, α-ketoglutaric acid, fumaric acid, dl-malic acid, sodium acetate, sodium pyruvate, and citric acid.

P. Mutant 83901T

Genetics

The genetic data for 83901T are very meager. Aberrant asci have been found in an outcross of 83901T to wild type. Mutant 83901T may therefore possess a chromosomal aberration (see McClintock, 1945).

For the biochemical studies two reisolates (83901T-R3-a and 83901T-R4-A) were used. These represent the two mutant spore-pairs of an ascus.

Biochemistry

Mutant 83901T will grow well but somewhat adaptively at 25° C. on minimal medium. At 36° C., its growth on minimal medium is highly variable; but, in general, it grows only slowly (Table 36).

Table 36. Growth of 83901T reisolates on minimal medium at 36° C.

Experiment	Reisolate	Duration of Growth (hrs.)	Mycelial Mass (mgs.)		
			1	2	3
1	R4-A	138	0.4	0.6	0.4
2	R4-A	99	21.3	10.5	--
3	R3-a	88	1.2	1.5	--
4	R3-a	90	12.3	1.1	--
5	R3-a	88	1.4	1.3	--
6	R3-a	84	1.0	2.7	--
7	R3-a	86	2.3	2.4	--
8	R3-a	98	1.7	2.2	--
9	R3-a	93	1.4	1.2	--
10	R3-a	96	0.9	10.8	--

Table 36. (Continued)

The tests were made in duplicate or triplicate, as indicated.

It is not known chemically what 83901T needs for maximal growth at 36° C. None of the known mixtures used in this study has been found to be active (reproducibly). All the component compounds of these known mixtures have been tested individually. None was active. Other compounds which are inactive, at least in the concentrations tested, include those listed in Table 37.

Table 37. Some additional compounds inactive for 83901T

adenosine	d-fructose
adenosine-3-phosphoric acid	d-galactose
β-alanine	d-glucosamine hydrochloride
alanylglycine	d-glucose
dl-α-amino-n-butyric acid	l-glutamine
l-arabinose	glutathione
ascorbic acid	glycine betaine hydrochloride
calcium hexose diphosphate	glycylglycine
citric acid	guanine hydrochloride
creatine	hemin
l-cysteine monohydrochloride	dl-homoserine
cytochrome-c	hypoxanthine
2,6-diaminopurine sulfate	inosine
N-dimethylaminoethanol	dl-leucylglycine
ethyl carbamate	d-mannose
formaldehyde	orotic acid
formic acid	d-ribose

Table 37. (Continued)

sodium acetate	xanthine
uracil	xanthosine
urea	l-xylose
uric acid	

In addition to the compounds which have been tested singly, some likely combinations of compounds have been tried. All were inactive. Some of these are listed in Table 38.

Table 38. Some combinations of compounds inactive for 83901T

d-glucose + d-fructose

dl-isoleucine + dl-valine

dl-isoleucine + dl-methionine + dl-threonine

p-aminobenzoic acid + dl-phenylalanine + l-tryptophane + l-tyrosine

adenine sulfate + l-arginine monohydrochloride

adenine sulfate + dl-methionine

adenine sulfate + guanine hydrochloride + hypoxanthine + uric acid + xanthine

Mutant 83901T will grow nonadaptively at 36° C. when supplied with various chemically undefined growth sources. Liver extract (Lilly) and yeast extract (Fleischmann type 300) were the richest sources found. The activity of this liver extract is shown in Table 39.

Table 39. Growth of 83901T-R4-A for 96 hours at 36° C.

Medium	Mycelial Mass (mgs.)
M	3.7
Lv (0.25 mg./ml.)	6.7
Lv (0.80 mg./ml.)	20.3
Lv (2.1 mg./ml.)	39.2
Lv (3.6 mg./ml.)	54.0
Lv (5.0 mg./ml.)	60.3

Lv = liver extract (Lilly).

Since the amount of liver extract required for maximal growth is rather high, an experiment was performed to see if 83901T utilizes the sucrose normally present in minimal medium at 15 mg./ml. The results in Table 40 show that the sucrose is necessary for growth.

Table 40. Growth of 83901T-R3-a for 90 hours at 36° C.

Supplement to Basal Medium	Mycelial Mass (mgs.) 1	2
none	trace	trace
sucrose (15 mg./ml.)	12.3	1.1
liver extract (5 mg./ml.)	1.2	1.2
sucrose (15 mg./ml.) + liver extract (5 mg./ml.)	31.6	30.1

The basal medium here is minimal medium without sucrose. Tests were made in duplicate, as shown.

A chromatopile of liver extract was developed in a solvent of five volumes n-propanol to two volumes 0.3 N NH_4OH. This was bioassayed

and a rather broad peak of activity was found at Rf 0.33. At Rf 0.71, the eluates depressed the growth of 83901T below the level obtained on minimal medium.

This active peak (Rf-range of 0.29 to 0.36) was extracted, and its activity was shown to be five times that of original liver extract (on a mg. dry mycelium per mg. source basis). An aliquot of this active fraction was hydrolyzed with 6 N HCl for 20 hours at 103° C. in a sealed tube. This was neutralized and tested for activity. The one test made showed no loss in activity.

For any future research on this problem the following observations may be helpful:

1. With an n-butanol-water partition at pH's 2.0, 7.0, and 12.0, the activity is in the aqueous phase.

2. When acetone is salted out from a 50% aqueous acetone solution by the addition of K_2CO_3, about 20% to 40% of the activity is found in the acetone layer.

3. The activity in liver extract can be extracted with 75% aqueous ethanol but not with absolute ethanol, when 50 ml. of extracting solvent is used per 500 mg. liver extract. When such extracts into 75% ethanol are treated with increasing amounts of Norit A (Braun), the recovered unadsorbed activities are those given in Table 41.

Table 41. Growth of 83901T-R3-a for 88 hours at 36° C. when supplied with unadsorbed fractions after treatment of the 75% aqueous ethanol extract with Norit A at different adsorption ratios.

Medium	Adsorption Ratio	Mycelial Mass (mgs.) 1	2
M	--	1.2	1.5
liver extract (Lilly)	--	33.1	33.5
alc. extr.	--	44.9	41.5
unads. fr. of alc. extr.	0.03	43.7	41.8
unads. fr. of alc. extr.	0.20	39.2	46.1
unads. fr. of alc. extr.	0.70	35.2	31.1
unads. fr. of alc. extr.	1.50	35.7	33.9
unads. fr. of alc. extr.	3.00	32.2	31.6
unads. fr. of alc. extr.	5.50	20.4	24.0
unads. fr. of alc. extr.	10.0	23.8	19.7

Adsorption ratio is mass charcoal/mass original liver extract. Each test is equivalent to original liver extract (Lilly) at 5 mg./ml. The tests were in duplicate, as indicated. Abbreviations: alc. extr. = extract into 75% aqueous ethanol; unads. fr. = unadsorbed fraction.

The above fraction unadsorbed at an adsorption ratio of 10.0 has been called fraction B. Maximal growth can be obtained with this fraction.

4. If this fraction B is treated with increasing amounts of Fuller's Earth, the recovered unadsorbed activities are those given in Table 42.

Table 42. Growth of 83901T-R3-a for 88 hours at 36° C. when supplied with unadsorbed fractions after treatment of fraction B with Fuller's Earth at different adsorption ratios.

Medium	Adsorption Ratio	Mycelial Mass (mgs.) 1	2
M	--	1.4	1.3
liver extract (Lilly)	--	23.2	23.7
Fraction B	--	19.3	24.9
unads. fr. of B	0.05	13.4	14.0
unads. fr. of B	0.25	21.8	24.2
unads. fr. of B	1.25	5.2	5.5
unads. fr. of B	6.25	3.9	3.5

Each test is equivalent to original liver extract at 5 mg./ml. Tests were in duplicate, as indicated. Abbreviation: unads. fr. = unadsorbed fraction.

Apparently most of the activity can be adsorbed onto Fuller's Earth.

IV. DISCUSSION

The discussion is in two parts: (A) a treatment of the particular types of mutants found, and (B) a general discussion.

A. The Types of Mutants Found

1. Pyrimidineless (C126 and C121)

Mutants C126 and C121 are clearly pyrimidineless mutants. Both were in this group of "unknowns" because they were originally in double-mutant strains---C126 and C127 (ser) in circle-16, and C121 and C122 (arg) in alpha-51. Double-mutant circle-16 grew only poorly and variably on the chemically defined mixture V A M-4 V-4, which contains the required compounds serine and cytidine. Double-mutant alpha-51 did not respond to the same mixture V A M-4 V-4, which contains the required compounds arginine and cytidine. The presence of inhibitors of these double mutants in V A M-4 V-4 accounts for these results.

Both C126 and C121 will utilize cytidine for maximal growth; and, as far as is known at present, they are not nutritionally different from some pyrimidineless mutants found earlier (H. K. Mitchell and Houlahan, 1947).

2. Serineless (C127, 47903, and 65004)

Mutant C127 was isolated from the double-mutant "unknown" circle-16 (see discussion of pyrimidineless mutants). Strains 47903 and 65004 were found among the "unknowns" presumably because they grow only in trace amounts on acid-hydrolyzed casein, which is the amino acid mixture commonly used in this type of work. They had not been tested on serine alone.

All three of these mutants will utilize dl-serine moderately well

and will respond to formic acid adaptively. Formaldehyde was shown to support adaptive growth of 47903, and adenine, methionine, tryptophane, lysine, and choline, in various combinations, will support poor to fair growth of 47903. If cytidine is supplied, adenine and tryptophane are stimulatory to the growth of the double-mutant circle-16 ($\underline{pyr}^{C126}$ $\underline{ser}^{C127}$). These stimulations are likely of the component mutant C127, not of C126.

Harrold (1951) showed that mutant T2269 will utilize formic acid. Harrold and Fling (1952) studied mutants C24 and T3207, both of which will utilize formic acid or formaldehyde. These investigators found that, under certain conditions, the compounds glycine, serine, adenine, choline, methionine, and histidine could be demonstrated to stimulate the growth of at least one of these mutants.

These are then six separate occurrences of mutants which will utilize formic acid for growth, and there are nine other compounds (adenine, choline, formaldehyde, glycine, histidine, lysine, methionine, serine, tryptophane) which are demonstrably stimulatory under at least some conditions. Since several of these compounds have been implicated in one-carbon fragment metabolism, it is not unreasonable to suppose that these mutants have this part of their metabolism genetically impaired, as suggested by Harrold and Fling (1952).

The responses of these mutants to these various compounds are given in Table 43.

As shown in Table 43, all of these formic acid-utilizing mutants except C24 will utilize serine. The mutant genes in these are rather arbitrarily designated <u>serineless</u> (<u>ser</u>), whereas the mutant gene of

C24 is called formicless (for).

Table 43. Responses of formic acid-utilizing mutants to various compounds.

	Response to Compound					
	Mutant					
Compound	C24	T2269	T3207	C127	47903	65004
adenine	+	0	+	+	+	0
choline	+	0	+	0	+	0
formaldehyde	+	0	+	0	+	0
formic acid	+	+	+	+	+	+
glycine	+	+	+	0	0	0
histidine	+	0	--	0	0	0
lysine	0	0	0	0	+	0
methionine	+	0	±	0	+	0
serine	--	+	+	+	+	+
tryptophane	0	0	0	+	+	0

Key to symbols: + = stimulatory at least under some conditions; ± = variable results; -- = not stimulatory in the tests which have been made; 0 = no information. The data for T2269 are from Harrold (1951), for C24 and T3207 are from Harrold and Fling (1952).

Reisolates of C127 and 65004 show considerable variability in their response to dl-serine. (see results section for data with C127.) Reisolates of 47903 have not been investigated in this regard. Harrold and Fling (1952) found that the reisolates of T3207 did not respond as well to serine or formic acid as did the parental culture T3207-A. Harrold (1951) reported similar behavior for reisolates of T2269. No explanation of this behavior was offered.

Such variability could be accounted for by genetic modification

of the expression of the particular ser gene, but any such modifiers have yet to be traced down by genetic methods. Such genetic modifications of expression has been reported in Neurospora (see, e.g., Haskins and H. K. Mitchell, 1952; M. B. Mitchell and H. K. Mitchell, 1952a). Some variability with respect to the utilization of the compound required is expected and is found with most biochemical mutants of Neurospora crassa, but the differential utilization found with these serineless mutants is of an order of magnitude different from the average.

It is attractive to speculate that there may be a compound which would, in many diverse genetic backgrounds, support maximal growth of one or more of the serineless mutants T2269, T3207, C127, and 65004, and that this compound is not dispensable to Neurospora for the reason of impermeability. The growth activities of serine, adenine, methionine, and these other stimulatory compounds could then be interpreted as sparing effects, if it was assumed that this indispensable compound participated catalytically in their biosyntheses. It can be suggested that the indispensable compound might be any one of several biochemically important compounds for which there have been no demonstrated requirements by Neurospora mutants. Four such compounds, which are also apparently involved in one-carbon fragment metabolism, are vitamin B12, folic acid, folinic acid, and adenine thiomethylriboside. With reference to this idea, it would be interesting to determine the permeability of Neurospora to these compounds.

The available genetic data (Table 44) indicate that $\underline{for}^{C24}$, $\underline{ser}^{47903}$, and $\underline{ser}^{65004}$ are at three different loci.

Table 44. Intercrosses between formic acid-utilizing mutants

	65004	47903	C127	T3207	T2269
C24	N	N	--	N*	S
T2269	S	S**	--	--	
T3207	S	S**	--		
C127	--	--			
47903	N				

*Shown by Harrold and Fling (1952). **Recently, reciprocals of these sterile intercrosses have been found to be fertile.

Key to symbols: N = nonallelic; -- = untested; S = all attempted crosses have been sterile or very infertile.

3. Aromaticless (C120)

Mutant C120 grows only poorly on the known mixture V A M-4 V-4, which contains the four aromatic compounds needed for growth. This explains the original classification of C120 as an "unknown" and illustrates again the importance of inhibitions of Neurospora mutants by metabolites ordinarily nontoxic.

In its utilization of phenylalanine plus tyrosine plus anthranilic acid (as a substitute for tryptophane) plus p-aminobenzoic acid for maximal growth, mutant C120 is similar to Neurospora mutant Y7655, which was found and investigated by Tatum (1949). Mutant Y7655 will utilize the cyclohexene derivative shikimic acid, however, whereas C120 will not. Davis has investigated the biosynthesis of aromatic compounds in Escherichia coli (see review by Davis, 1952). He has placed shikimic acid on the metabolic path to the aromatic compounds tyrosine, phenylalanine, tryptophane, p-aminobenzoic acid, p-hydroxybenzoic acid, and a sixth unknown factor. If shikimic acid is an intermediate in aromatic biosynthesis in Neurospora as it appears to be in E. coli, mutant C120 is genetically blocked after it in the metabolic path. Davis has found mutants of E. coli which accumulate shikimic acid, and it is not unreasonable to expect that mutant C120 may accumulate it, at least under certain conditions.

Arginine and lysine are slightly stimulatory to the growth of C120. The mechanisms of these stimulations are not understood, but it is interesting to note that Davis (1952) has observed that methionine plus lysine can largely replace a requirement for p-hydroxybenzoic acid in quintuple aromatic auxotrophs of E. coli. He has suggested that

p-hydroxybenzoic acid participates in lysine and methionine biosyntheses, at least in E. coli. The requirement for p-hydroxybenzoic acid by these strains studied by Davis is, under ordinary conditions, a relative one. It is an absolute one, however, if l-aspartic acid is added to the medium. In addition, Davis has found an absolute requirement for a sixth unknown factor if these quintuple aromatic auxotrophs are grown at pH 7.5. This factor is found in wild-type E. coli filtrate. Although it does not seem likely, since the requirements of C120 for tryptophane and p-aminobenzoic acid are relative under usual conditions of culture, it would be at least interesting to see if further requirements could be revealed with altered environments.

The genetic studies with C120 indicate that aro^{C120} is a useful marker about four units from a centromere---perhaps the centromere of linkage group G (see results). If aro^{C120} is in linkage group G, it is apparently the second biochemical marker there. Barratt and Garnjobst (1949) found a biochemical marker (Y2492) in Lindegren's group II, which is apparently identical with group G.

Recently Metzenberg (1952) has isolated another aromaticless Neurospora mutant, which is nutritionally similar to mutant C120 in its lack of growth response to shikimic acid.

4. Argininеless (C122)

Mutant C122 was isolated from the double mutant alpha-51, which would not grow on known mixture V A M-4 V-4 (see discussion of pyrimidineless mutants). At present C122 is not known to be different from other argininеless mutants which will utilize ornithine (Srb, 1947). The sparing effect of lysine for C122 cannot be explained at this time. This effect of lysine is quite in contrast to its inhibitory

effect for some arginineless mutants studied by Srb (1947).

5. Asparagineless (C123 and 67603T)

These were found in the "unknown" category simply because asparagine was not present in the known mixtures used in the preliminary investigations.

Both of these mutants grow nonadaptively when supplied with l-asparagine. Mutant 67603T, however, will grow very well adaptively on minimal medium, whereas C123 will not. Qualitative tests of about 20 reisolates of 67603-A and about 45 reisolates of C123-A from outcrosses to the same wild-type strain (5297-a) showed that the reisolates with asg^{67603} grew much more on minimal medium than did the reisolates with asg^{C123}. This suggested that the alleles asg^{C123} and asg^{67603} are different. It is still possible, however, that the differences in growth behavior between the two parental strains are due to rather complex modification by genes at other loci.

The genes asg^{C123} and asg^{67603} are either allelic with or very close to asg^{1A7}, the mutant gene of the asparagineless mutant investigated by Surber (1951). Mutant 1A7-a is similar to C123-A in its growth behavior on minimal medium.

The asg locus is on the pab^{1633}-containing arm of linkage group E.

These asg genes should be useful in an investigation of asparagine biosynthesis in Neurospora crassa.

6. Slow-grower (C125)

The name "slow-grower" is used here to mean an "indispensable" (or relatively "indispensable") mutant which grows slowly on minimal

medium.

This mutant was isolated from a double-mutant "unknown." It will grow only adaptively on any medium tested. This adaptive growth is apparently slightly stimulated by Neurospora autolysate. Although C125 may later be shown to be "dispensable," it is at present classifiable only as a relatively "indispensable" slow-growing mutant.

These slow-growers apparently make up a large share of the mutants of Neurospora crassa (Lein, H. K. Mitchell, and Houlahan, 1948), but in the past there has been a rather large selection against this category. An interesting example of a slow-grower is the Neurospora strain "poky," whose mutant character is inherited at least predominantly through the protoperithecial parent (M. B. Mitchell and H. K. Mitchell, 1952b). Recently some slow-growers, whose mutant characters are inherited in typical Mendelian fashion, have been investigated biochemically by H. K. Mitchell, Tissieres, and M. B. Mitchell (1952).

7. Homoserineless (35709)

Mutant 35709 was isolated from a double-mutant "unknown" of genotype $hmsr^{35709}$ sl^{C125} (slow-grower). The slow growth of this double mutant could account for its former position in the "unknown" category.

The gene $hmsr^{35709}$ is either allelic with or close to $hmsr^{51504}$. (See results section for references.) Both 35709 and 51504 will utilize homoserine for maximal growth, but in other aspects of their growth behavior they are different (see results). It is not known whether these differences in growth behavior stem from genetic modification at other loci or are due simply to a difference between $hmsr^{35709}$ and $hmsr^{51504}$.

8. Para-aminobenzoicless (36111)

There is no obvious reason why 36111 was found in the "unknown" group. The gene pab^{36111} is probably allelic with pab^{1633}. Both 36111 and 1633 will grow maximally on p-aminobenzoic acid. Mutant 36111 will utilize p-nitrobenzoic acid for adaptive growth as will 1633 (Tatum and Beadle, 1942). Mutant C120 (aro), which has been discussed previously, will utilize p-nitrobenzoic acid in place of its p-aminobenzoic acid requirement.

Egami, Ebata, and Sato (1951) obtained enzymatic reduction of the aromatic nitro group of chloromycetin to an amino group. They used cell-free extracts from Streptococcus haemolyticus. Identity between this system and the nitrite reductase system (Yamagata, 1939) was suggested by these workers. It then seems reasonable to postulate that Neurospora can reduce p-nitrobenzoic acid to p-aminobenzoic acid. The lag period before growth on p-nitrobenzoic acid could then possibly be ascribed to the time taken for this enzyme system to be formed and for it to produce sufficient p-aminobenzoic acid.

9. Aceticless (37812)

Mutant 37812 is peculiar in its lack of growth on liquid minimal medium supplemented with any of the chemically defined or undefined mixtures used in this work except enzymatically hydrolyzed casein. The activity of enzymatically hydrolyzed casein is probably ascribable to its content of acetic acid (see results), which alone will support fine growth of 37812 on agar slants. Acetic acid has not been tested in a liquid medium. The inactivity of the complex growth sources other than enzymatically hydrolyzed casein has not been explained.

Other mutants of Neurospora crassa have been found to utilize acetic acid (Lein and Lein, 1950; Lein, Appleby, and Lein, 1951; Strauss, 1952, Lein and Lein, 1952). It is not known at present how mutant 37812 is related to these.

10. Methionineless (C124)

Mutant C124 was isolated from the double mutant of genotype meth^{C124} aspt^{44303T}. This double mutant was stimulated by methionine but would not grow maximally on it alone. Mutant C124 will grow maximally on methionine but is not stimulated by homocysteine thiolactone hydrochloride. Presumably then the methylation of homocysteine is genetically blocked in this strain. There are many separate occurrences of such mutants in Neurospora crassa, and several different loci are involved (Gershowitz, 1952).

11. Asparticless (44303T)

Aspartic acid, glutamic acid, homoserine, and probably several other amino acids will stimulate the growth of 44303T. In this respect, 44303T is similar to the mutants 32212 and 47305 studied by Fincham (1950) and interpreted by him to be "amination-deficient." Fincham (1951) was unable to find any l-glutamic acid dehydrogenase activity in mutants 32212 and 47305.

Mutant 44303T, however, is inhibited by dl-alanine, an amino acid which is active for the growth of 32213 and 47305. Strain 44303T also differs from 32213 and 47305 in its failure to grow maximally on any one amino acid. It appears then that 44303T is different from Fincham's mutants. More work will be needed to reveal the nature of the genetic

block in 44303T.

12. Leucineless (45208T)

Mutant 45208T is strongly inhibited by isoleucine and phenylalanine. These inhibitions may account for its former place in the "unknown" group, since it may have been tested on amino acid mixtures with high concentrations of these inhibitors.

This mutant will grow maximally and nonadaptively on leucine at 25° C. At 36° C., however, its growth is poor on leucine. Liver extract (Lilly), which is an excellent chemically undefined source for growth at 25° C., supports no growth at 36° C. It is not known whether this temperature-sensitivity is associated with leu^{45208T} or is effected through the action of genes at other loci.

The relationship of 45208T to the Neurospora leucineless mutants studied by Regnery (1947) is not known.

13. Succinicless (46005 and 46403)

Lewis (1948) investigated Neurospora mutants 35402, 37602, 39311, and 39404. These will grow maximally when supplied with succinic acid or some related compounds. Mutants 46005 and 46403 are similar to these four strains with respect to the compounds utilized for growth. Lewis, however, reported that glutamic acid alone or aspartic acid alone would suffice for growth, and he therefore ascribed the activity of acid-hydrolyzed casein to its content of these two amino acids. In this investigation, however, neither aspartic acid nor glutamic acid nor a mixture of the two was very active. At any rate, they did not account for the high activity of acid-hydrolyzed casein. Asparagine or glutamine, however, will support good growth of 46005 or 46403. It is

not understood why these are so much more active than aspartic acid and glutamic acid.

Surber (1951) found that fructose would support fair growth of a succinicless mutant she was investigating. At this time it is difficult to explain this fact and, at the same time, all the other information on the growth behavior of these succinicless mutants.

14. Methionine-adenine-cystineless (65108)

Mutant 65108 was isolated from a double-mutant "unknown" of genotype mac^{65108} un^{128T}. Methionine plus adenine plus cystine will support nonadaptive maximal growth of 65108. Cystine was stimulatory under the conditions used (see results), but it is quite possible that maximal growth can be obtained with just adenine plus methionine. Methionine alone, but neither adenine alone nor cystine alone, nor adenine plus cystine, will support good adaptive growth. Homocysteine thiolactone hydrochloride will not substitute for methionine. Mutant C24 (Harrold and Fling, 1952; see discussion of serineless mutants) is similar to 65108 in that it will grow well on adenine plus methionine. Formic acid, however, is utilized by C24 but not by 65108. The growth of C24 is stimulated by adenine alone but not by methionine alone, in contrast to 65108. Furthermore, the gene mac^{65108} is about four units from alb_2^{15300} in linkage group A, whereas for^{C24} is apparently either in group F or group G (M. B. Mitchell and Pittenger, 1952).

The relative requirement of 65108 for adenine is probably related to the inhibition by guanine of the adaptive growth on methionine. It is not known, though, whether or not added adenine will overcome this

guanine inhibition. Smith and Schlenk (1952a and 1952b) found that adenine thiomethylriboside is accumulated by yeast if the medium is supplemented with a large amount of methionine. Although adenine thiomethylriboside, even with added homocysteine and choline, is unable to replace the methionine requirement of Photobacterium phosphoreum, Lactobacillus casei ϵ, and Lactobacillus arabinosus (Schlenk and Gingrich, 1944), it is still possible that it might suffice for mutant 65108. None has been available for testing.

15. Unknown (C128T, 83901T)

Only a very preliminary investigation has been made of C128T. An interesting property of this mutant is its temperature-sensitivity. At 36° C., it has wild-type growth behavior. At 25° C., it grows only adaptively on minimal medium, and its growth is stimulated by Neurospora autolysate.

Mutant 83901T, on the other hand, grows more at 25° C. than at 36° C. on minimal medium. Liver extract and some other complex mixtures will effect maximal growth at 36° C. It cannot be said at the present time that there is in liver extract a compound which alone will support maximal growth. Many compounds and combinations of compounds have been examined and shown to be inactive. It therefore seems likely that this is a mutant which is new to Neurospora work. In view of past experience, however, this may not be the case.

B. General

It is interesting but certainly not surprising to note that five of the 16 "unknowns" investigated were mutant for two genes. It was, of course, far easier to elucidate the growth requirements of these double-mutant "unknowns" after they had been genetically resolved into their component single-gene mutants.

This investigation has revealed several apparently new mutants of Neurospora crassa. Mutant C120 is apparently the first aromaticless Neurospora mutant which will not utilize shikimic acid. One or more of the formic acid-utilizing mutants C127, 47903, and 65004 may be genetically different from all those studied earlier (C24, T2269, and T3207). At any rate, the brief genetic study made in this investigation has demonstrated no cases of allelism. The asparagineless mutants (C123 and 67603) are relatively new in Neurospora work. The asparagineless mutant (1A7) investigated by Surber (1951) was, as far as is known by the author, the first one found. Neither 44303T nor 65108 appears to be like any other reported mutants. It may be suggested, however, that some of the strains previously classified as methionineless and apparently blocked after homocysteine may be of the same type as 65108.

In several cases (circle-16, alpha-10, alpha-51, 47903, and 65004, for example) these "unknowns" would not grow or grew only poorly on complicated chemically defined mixtures which contained the compound or compounds required. This was presumably due to the presence of inhibitors in these known mixtures. Such inhibitions are fairly common among Neurospora mutants (see, e.g., Doermann, 1944). Horowitz and

Leupold (1951) have not ascribed these inhibition phenomena to additional gene functions. They have interpreted them as being "inherent in the mechanism of utilization of the exogenously provided growth substance." Their argument is based on the observation that these inhibitions usually are not locus-specific.

Of the 21 single-gene* mutants obtained from these "unknowns," all except C125 may be reasonably classified as "dispensable." Of the 20 "dispensable" strains, 12 may be classified as resulting from mutations of "unifunctional" genes, by the criteria of Horowitz and Leupold (1951). Of the remaining eight, two (C128T and 83901T) are still "unknowns" and one (C120) can be reasonably interpreted as having a multiple requirement resulting from a single metabolic block. Mutants 44303T (aspt), 65108 (mac), C127 (ser), 65004 (ser), and 47903 (ser) are the remaining five. If the original cultures and the reisolates of these are considered, then none responds consistently and maximally to any single compound which has been tested. Mutant 47903 (ser) may not belong in this group. It is included here, however, since it is suspected that its reisolates may be as variable in their utilization of serine as are the reisolates of T2269 (ser), T3207 (ser), C127 (ser), and 65004 (ser). All that can be said is that the present information does not warrant the classification of the wild-type alleles of the mutant genes in these mutants as "unifunctional," as defined by Horowitz and Leupold (1951).

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*The evidence for the single-gene nature of some of these is meager.

BIBLIOGRAPHY

1. Barratt, R. W., and L. Garnjobst, (1949), Genetics, 34, 351.
2. Beadle, G. W., and E. L. Tatum, (1945), Am. J. Bot., 32, 678.
3. Bonner, D., E. L. Tatum, and G. W. Beadle, (1943), Arch. Biochem., 3, 71.
4. Davis, B. D., (1950), Experientia, VI, 141.
5. Davis, B. D., (1952), II^{e} Congrès International de Biochimie: Symposium sur le Métabolisme Microbien, p. 32.
6. Doermann, A. H., (1944), Arch. Biochem., 5, 373.
7. Egami, F., E. Mituo, and R. Sato, (1951), Nature, 167, 118.
8. Emerson, S. E., (1952), personal communication.
9. Fincham, J. R. S., (1950), J. B. C., 182, 61.
10. Fincham, J. R. S., (1951), J. Gen. Microbiol., 5, 793.
11. Fling, M. and N. H. Horowitz, (1951), J. B. C., 190, 277.
12. Gershowitz, H., (1952), personal communication.
13. Gordon, M., F. A. Haskins, and H. K. Mitchell, (1950), Proc. Nat. Acad. Sci., 36, 427.
14. Harrold, C. E., (1951), unpublished results.
15. Harrold, C. E., and M. Fling, (1952), J. B. C., 194, 399.
16. Haskins, F. A., and H. K. Mitchell, (1952), The American Naturalist, LXXXVI, 231.
17. Horowitz, N. H., and U. Leupold, (1951), Cold Spring Harbor Symposia on Quantitative Biology, XVI, 65.
18. Houlahan, M. B., G. W. Beadle, and H. G. Calhoun, (1949), Genetics, 34, 493.

19. Lein, J., D. C. Appleby, and P. S. Lein, (1951), Arch. Biochem. and Biophys., 34, 72.

20. Lein, J., and P. S. Lein, (1950), J. Bact., 60, 185.

21. Lein, J., and P. S. Lein, (1952), Proc. Nat. Acad. Sci., 38, 44.

22. Lein, J., H. K. Mitchell, and M. B. Houlahan, (1948), Proc. Nat. Acad. Sci., 34, 435.

23. Lewis, K. W., (1948), Am. J. Bot., 35, 292.

24. Lindegren, C. C., and G. Lindegren, (1939), Genetics, 24, 1.

25. Markert, C. L., (1952), Genetics, 37, 339.

26. Mauron, J., (1950), unpublished results.

27. Mauron, J., and G. R. Dubes, (1950), unpublished results.

28. McClintock, B., (1945), Am. J. Bot., 32, 671.

29. Metzenberg, R., (1952), unpublished results.

30. Mitchell, H. K., (1951), unpublished results.

31. Mitchell, H. K., (1951), Vitamins and Hormones, VIII, 127.

32. Mitchell, H. K., and F. A. Haskins, (1949), Science, 110, 278.

33. Mitchell, H. K., and M. B. Houlahan, (1947), Fed. Proc., 6, 506.

34. Mitchell, H. K., A. Tissieres, and M. B. Mitchell, (1952), personal communication.

35. Mitchell, M. B., (1952), personal communication.

36. Mitchell, M. B., and H. K. Mitchell, (1952a), Proc. Nat. Acad. Sci., 38, 205.

37. Mitchell, M. B., and H. K. Mitchell, (1952b), Proc. Nat. Acad. Sci., 38, 442.

38. Mitchell, M. B., and T. H. Pittenger, (1952), unpublished results.

39. Mitchell, M. B., T. H. Pittenger, and H. K. Mitchell, (1952), Proc. Nat. Acad. Sci., 38, 569.

40. Regnery, D. C., (1947), Ph.D. Dissertation, California Institute of Technology.

41. Schlenk, F., and W. D. Gingrich, (1944), Fed. Proc. 3, 63.

42. Smith, R. L., and F. Schlenk, (1952a), Arch. Biochem. and Biophys., 38, 159.

43. Smith, R. L., and F. Schlenk, (1952b), Arch. Biochem. and Biophys., 38, 167.

44. Srb, A. M., (1947), Ph.D. Dissertation, Stanford Univ.

45. Strauss, B. S., (1952), Arch. Biochem. and Biophys., 36, 33.

46. Surber, H., (1951), unpublished results.

47. Tatum, E. L.,(1949), Fed. Proc., 8, 511.

48. Tatum, E. L., and G. W. Beadle, (1942), Proc. Nat. Acad. Sci., 28, 234.

49. Teas, H. J., (1946), M.A. Dissertation, Stanford Univ.

50. Teas, H. J., (1947), Ph.D. Dissertation, California Institute of Technology.

51. Teas, H. J., (1952), personal communication.

52. Teas, H. J., N. H. Horowitz, and M. Fling, (1948), J. B. C., 172, 651.

53. Westergaard, M., and H. K. Mitchell, (1947), Am. J. Bot.,34, 573.

54. Yamagata, S., (1939), Acta Phytochimica (Japan), 11, 145.