GENETICAL AND BIOCHEMICAL STUDIES OF HISTIDINE-REQUIRING MUTANTS OF NEUROSPORA CRASSA. I. CLASSIFICATION OF MUTANTS AND CHARACTERIZATION OF MUTANT GROUPS[1]

BROOKE B. WEBBER[2] AND MARY E. CASE

Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut

Received May 19, 1960

SEVEN histidine-requiring mutants of Neurospora were described by HAAS, MITCHELL, AMES and MITCHELL (1952) and placed in four distinct genetic groups (subsequently designated *hist-1* through *hist-4* by BARRATT, NEWMEYER, PERKINS, and GARNJOBST 1954). One of these groups *(hist-3)* was shown to comprise mutants of at least two distinct biochemical classes. MATHIESON and CATCHESIDE (1955) also reported studies of a *hist-2* mutant, K-12, a *hist-3* mutant, K-26, and a distinct new mutant, K-34.

The classification by heterocaryon tests of 704 additional histidine mutants which were derived in genetically closely related wild-type strains from three filtration-concentration experiments is described here. As the classification proceeded, new groups were found which had not been described previously in the literature. Representatives of the various groups were studied physiologically and genetically to characterize the groups with respect to their positions in the histidine biosynthetic scheme. The classification and characterization of various groups will be described here in detail.

MATERIALS AND METHODS

The 704 histidine-requiring mutants were obtained from three filtration-concentration experiments (Y152, Y155, and Y175) from untreated and X-irradiated macroconidia. These mutants are designated by experiment number (Y....) and arbitrary mutant number (M....). In these experiments three wild-type strains were used, namely 74A (from DR. PATRICIA ST. LAWRENCE) and two very closely related strains, 5.5A and 3.1a (CASE and GILES 1958). The X-ray dose (10,000r to 36,000r in different experiments) was administered at 4000r/minute with a General Electric Maxitron 250 unit operated at 250 kv and 30 milliamperes with a 1 mm aluminum filter. Other strains used in the genetic analyses are listed in Table 1.

[1] From a dissertation presented by the senior author in partial fulfilment of the degree of Doctor of Philosophy in the Graduate School of Yale University. This investigation was supported in part by a Summer Research Fellowship from Yale University, by a Junior Sterling Scholarship, 1957–1958, from Yale University, by a National Science Foundation predoctoral fellowship, 1958–1959, and by the Atomic Energy Commission under contract AT(30–1)–872 administered by DR. NORMAN H. GILES.

[2] Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

TABLE 1

Mutant strains used in genetic analyses

Isolation number	Locus designation(s)*	Phenotype
C84	*hist-1*	requires histidine
C94	*hist-2*	requires histidine
C140	*hist-3*	requires histidine
T1710	*hist-3*	requires histidine
C141	*hist-4*	requires histidine
K-26	*hist-3*	requires histidine
K-34		requires histidine
10575	*tryp-1*	requires tryptophane
74-YU938-1a	*arg-3 nic-2*	requires arginine and nicotinamide
74-OR14-6A	*ad-5 nic-2*	requires adenine and nicotinamide
5531A	*pan-1*	requires pantothenic acid
F12 (74A-Y155-M39)	*ad-4*	requires adenine

* Information concerning the first five histidine strains, *tryp-1*, and *pan-1* is listed (with prior references) in BARRATT *et al.* (1954). Information on strains K-26 and K-34 is from MATHIESON and CATCHESIDE (1955). Information concerning the *ad-4* isolate can be found in GILES *et al.* (1957).

Heterocaryon complementation tests were carried out in the manner previously described by DE SERRES (1956). The over plating tests used in the genetic analyses were performed essentially in the manner described by NEWMEYER (1954).

Classification of mutants by heterocaryon tests

Initially 50 mutants from experiment Y155 (Table 2) were tested in all combinations by pairs in heterocaryon tests. Mutants which were mutually noncomplementary were placed in the same group, and a tester was arbitrarily selected to represent each group. Ultimately the 704 histidine mutants were divided into six major groups by further heterocaryon tests.

Since the previously described mutants (K-34 and representatives of groups *hist-1* through *hist-4*) were not compatible with the newly acquired histidine mutants, crosses were made with appropriate wild-type strains (74A or 3.1a) to introduce appropriate heterocaryon compatibility factors. With the heterocaryon-compatible strains thus obtained, tests were then made with representatives of each of the six heterocaryotically determined groups. Four of the heterocaryon groups were shown to be noncomplementary (presumably allelic) with the previously designated *hist-1*, *hist-2*, *hist-3*, and K-34 mutants. However, *hist-4* had no equivalent among the new groups by these tests and appears to be a distinct group.

The results of these heterocaryon complementation tests are summarized in Table 2 which indicates the classification of the mutants from the three different filtration experiments. Seven major groups of histidine-requiring mutants have thus been detected. Groups *hist-1* through *hist-4* have been previously designated by BARRATT *et al.* (1954). The additional new groups designated here are *hist-5*, and *hist-7*, with K-34 placed in the *hist-6* group.

TABLE 2

Classification of histidine mutants from three filtration concentration experiments

Experiment number	Wild type used	*hist-1*	*hist-2*	*hist-3*	*hist-4*	*hist-5*	*hist-6*	*hist-7*	Total
Y152	74A	7	8	8	0	15	13	2	53
Y155	74A	9	22	22	0	17	16	0	86
Y175	5.5A	39	41	82	0	80	76	10	328
Y175	3.1a	18	54	64	0	42	59	0	237
	Total	73	125	176	0	154	164	12	

Biochemical characteristics

Accumulation products of single histidine mutants: Physiological experiments were carried out in order to determine the biosynthetic step which is blocked in each of the new groups of mutants. The results of cross-feeding experiments in which mutants were incubated on minimal agar petri plates on opposite sides of a permeable cellophane membrane indicated that no compounds stimulatory to the growth of any histidine mutants were excreted by other histidine mutants. These results were in agreement with those of HAAS *et al.* (1952) in attempts to stimulate histidine mutants with culture filtrates and with synthetic supplements.

The evidence of AMES, MITCHELL and MITCHELL (1953) concerning the nature of the imidazole derivatives accumulated by representatives of various mutant groups and the biochemical evidence reviewed by AMES (1957) indicate the position of the biochemical blocks in certain of the groups. *hist-1* mutants are deficient for imidazoleglycerol phosphate dehydrase activity and accumulate imidazoleglycerol in growth medium. The *hist-2* mutant is blocked prior to imidazoleglycerol phosphate, as no imidazoles are accumulated in the medium during growth. *hist-3* mutants are heterogeneous, with C140 blocked early (accumulating no imidazoles) and T1710 accumulating L-histidinol and deficient for histidinol dehydrogenase activity. The *hist-4* mutant was shown by AMES (1957) to exhibit less than two percent of wild-type activity for histidinol phosphate phosphatase and to accumulate both imidazoleglycerol and imidazoleacetol in the medium during growth. The *hist-6* mutant, K-34 of MATHIESON and CATCHSIDE (1955), was found to accumulate no imidazoles and, hence, was placed prior to imidazoleglycerol phosphate in the histidine biosynthetic scheme.

PAULY's (1904) tests for imidazoles and BRATTON-MARSHALL (1939) tests for diazotizable amines were carried out to determine whether imidazoles or diazotizable amines were accumulated by representatives of the mutant groups and to determine the biochemical characteristics of each of the new histidine mutant groups. For this purpose, representatives of each group were grown in 2 × 15 cm test tubes on 5 ml each of Fries' liquid medium supplemented with 25 micrograms/ml of L-histidine HCl. With this medium the histidine supply is limiting, and the exogenous histidine supply is usually exhausted within seven days. The mycelial pads were squeezed dry and autoclaved briefly in 0.5–1.0 ml of water. The resulting liquid was spotted on Whatman No. 1 filter paper and

developed as an ascending chromatogram with either the acidic or basic solvent of HAAS *et al.* (1952). Spots of imidazole derivatives were detected by spraying with Pauly reagents as described by MATHIESON and CATCHSIDE (1955). The three most conspicuous imidazole derivatives, detected at approximately the Rf's indicated by AMES *et al.* (1953) and compared with synthetic histidinol and histidine or with accumulant spots from known mutants, were imidazoleglycerol, imidazoleacetol, and histidinol. In some samples light spots of unused histidine were also found. The phosphate derivatives of these compounds were not observed. Separate chromatograms were tested for diazotizable amines by modification of the Bratton-Marshall test in which the paper is sprayed first with 0.2 percent sodium nitrite in 0.1 N HCl, dried, and then sprayed with 0.2 percent N(1-naphthyl)-ethylene diamine HCl in 95 percent ethanol. The filtrates from the above cultures were also tested directly by Pauly tests as described by AMES and MITCHELL (1955) and by Bratton-Marshall tests as described by STEWART and SEVAG (1952).

The results of Pauly tests and Bratton-Marshall tests of filtrates from single histidine mutants are presented in Table 3. These results are consistent with those of previous workers although additional groups are studied here, and the

TABLE 3

Compounds accumulated in growth medium by representative mutants from the seven histidine groups

Mutant group	Representative mutants	Tests of whole filtrates		Chromatogram tests				Position of block in biosynthetic scheme
		Pauly	Bratton-Marshall	Bratton-Marshall	Imidazoleglycerol	Imidazoleacetol	Histidinol	
Wild type	74A	—	—	—	—	—	—	none
hist-1	Y155-M302	+	—	—	+	—	—	between imidazoleglycerol and imidazoleacetol
hist-2	Y152-M43	—	—	—	—	—	—	before imidazoleglycerol
hist-3 (nonacc)*	C140 Y152-M111 Y193-M16	—	—	—	—	—	—	before imidazoleglycerol
hist-3 (acc)†	T1710 Y155-M234 Y155-M261	+	—	—	—	—	+	after histidinol
hist-4	C141	+	—	—	+	+	—	between imidazoleacetol and histidinol
hist-5	Y152-M108	+	—	—	+	+	—	between imidazoleacetol and histidinol
hist-6	Y152-M105	—	+	+	—	—	—	before imidazoleglycerol
hist-7	Y152-M31	—	+	+	—	—	—	before imidazoleglycerol

* Nonaccumulator.
† Accumulator.

information from Bratton-Marshall tests is new. Certain *hist-3* mutants characteristically accumulate histidinol while others consistently accumulate no detectable imidazoles or diazotizable amines. The two groups of mutants which accumulate no Pauly or Bratton-Marshall positive compounds are the *hist-3* nonaccumulators and *hist-2* mutants. There are two groups, *hist-6* and *hist-7*, which accumulate only a diazotizable amine. *hist-1* mutants accumulate imidazoleglycerol in both the medium and mycelium. *hist-4* and *hist-5* mutants accumulate imidazoleglycerol and imidazoleacetol. *hist-4* has been shown by AMES (1957) to be deficient for histidinol phosphate phosphatase, hence the *hist-5* mutants may tentatively be assumed to lack imidazoleacetol phosphate transaminase activity. One class of *hist-3* mutants (deficient for histidinol dehydrogenase) accumulates histidinol.

Accumulation studies with double mutants: In tests for imidazole accumulation, HAAS *et al.* (1952) used double mutants, each containing two different histidine-requiring genes. A double mutant is expected to exhibit accumulation characteristics similar to those of the earlier of the two incorporated mutants in the biosynthetic scheme, and so the relative orders of pairs of genes in the histidine biosynthetic scheme may be tested by this means. Experiments were undertaken using this technique with Pauly and Bratton-Marshall tests in an attempt to establish the relative order of the early mutants which do not accumulate imidazoles (*hist-2*, *hist-3* nonaccumulators, *hist-6*, and *hist-7*) and to repeat the observations of HAAS *et al.* (1952) on *hist-3* accumulators and nonaccumulators with newly acquired *hist-3* mutants.

Progeny were obtained from appropriate crosses and double mutants selected by heterocaryon tests. The double mutants were grown on limiting histidine as described above, and the filtrates were tested by Pauly and Bratton-Marshall tests. The results of these tests for accumulants of double mutants are reported in Table 4.

TABLE 4

Tests of filtrates for compounds accumulated by double histidine mutants. (See Table 3 for controls, i.e., tests of filtrates of single mutants)

Genetic composition of double mutant strains	Imidazoles (Pauly tests)	Diazotizable amines (Bratton-Marshall tests)	Indicated order of two reactions blocked
hist-2 hist-7	—	—	*hist-2* before *hist-7*
hist-2 hist-1	—	—	*hist-2* before *hist-1*
hist-3 (Y152-M111) *hist-7*	—	—	*hist-3* before *hist-7*
hist-3 (Y152-M111) *hist-6*	—	—	*hist-3* before *hist-6*
hist-3 (Y152-M111) *hist-1*	—	—	*hist-3* before *hist-1*
hist-3 (Y152-M111) *hist-4*	—	—	*hist-3* before *hist-4*
hist-3 (Y193-M16) *hist-1*	—	—	*hist-3* before *hist-1*
hist-7 hist-1	—	+	*hist-7* before *hist-1*
hist-7 hist-5	—	+	*hist-7* before *hist-5*
hist-7 hist-4	—	+	*hist-7* before *hist-4*
hist-7 hist-3 (Y155-M261)	—	+	*hist-7* before *hist-3*
hist-6 hist-1	—	+	*hist-6* before *hist-1*
hist-6 hist-4	—	+	*hist-6* before *hist-4*

This evidence indicates that *hist-3* accumulators and nonaccumulators are blocked at different positions in the histidine biosynthetic scheme. The nonaccumulators (Y152-M111 and Y193-M16) are blocked before *hist-1* while evidence from doubles containing Y152-M111 indicates a position before *hist-6* and *hist-7* for this nonaccumulator. Y155-M261 appears from this evidence to be located no earlier than *hist-1* while the fact that it accumulates only histidinol (Table 3) places it in the terminal step.

The earliest genetic blocks, exhibited by *hist-3* nonaccumulators and *hist-2* mutants, result in a lack of accumulation of imidazoles and of diazotizable amines. The relative order of the two groups cannot be determined by present tests. Next in the biosynthetic scheme are the *hist-6* and *hist-7* mutants, which accumulate a diazotizable amine. Again it is impossible to determine the relative order of these two groups. The order of the remaining mutant groups is *hist-1*, *hist-5*, *hist-4* and *hist-3* accumulators, with the assignment of *hist-5* tentative, as discussed above. The order of these mutant groups in the biosynthetic scheme for histidine is summarized in Figure 1.

Genetic studies

The purpose of genetic studies with the histidine mutants is to confirm the identity of representatives from each of the seven major physiological groups described above and to test whether two groups of mutants, which appear on the basis of heterocaryon complementation tests to be dissimilar, actually might be allelic mutants exhibiting interallelic complementation (GILES, PARTRIDGE and NELSON 1957). Of special interest in this respect are the pairs of groups which exhibit indistinguishable accumulation characteristics. The determination of the genetic position of groups previously undetected may also facilitate the use of

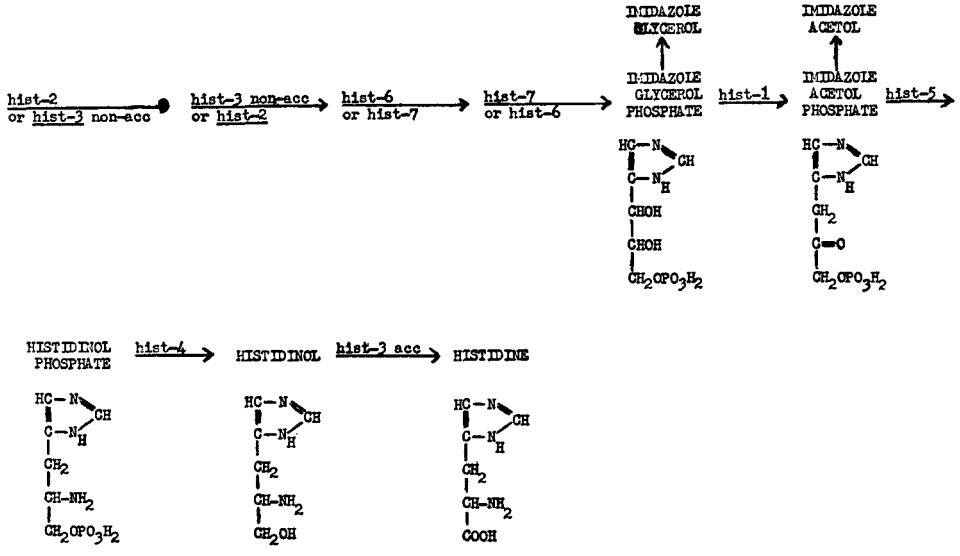

FIGURE 1.—Placement of mutant groups in the histidine biosynthetic scheme.

these mutants as markers for later crossing experiments. The genetic information concerning these mutants is listed in Table 5 and will be discussed for each group in the following paragraphs.

hist-1: This group was first assigned to linkage group V by HAAS *et al.* (1952). GILES (1955) reported evidence leading to the following order and map distances (in parenthesis) for certain linkage group V markers: centromere (10) isoleucinevaline (*iv-1*) (10) *hist-1* (7) inositol (*inos*) (3) para-aminobenzoic acid

TABLE 5

Genetic analysis for linkage relationship of histidine groups

Cross no.	Isolation numbers and genetic constitution of strains crossed	Estimate of viable spores plated on selective medium	Supplement in selective medium	Colonies in selective medium	Percent *hist*+ isolates*
1	Y152-M43 (*hist-2*)	2080	arginine	69	3.3
	74-YU938-1a (*arg-3 nic-2*)	2080	nicotinamide	41	2.0
		2080	none	0	
2	Y152-M14 (*hist-2*)	3770	arginine	139	3.7
	74-YU938-1a (*arg-3 nic-2*)	3770	nicotinamide	77	2.0
		3770	none	6	
3	Y193-M16 (*hist-3* nonacc)	7500	arginine	95	1.3
	74-YU938-1a (*arg-3 nic-2*)	7500	nicotinamide	213	2.8
		7500	none	9	
4	Y155-M261 (*hist-3* acc)	2960	arginine	68	2.3
	74-YU938-1a (*arg-3 nic-2*)	2960	nicotinamide	95	3.2
		2960	none	1	
5	C140 (*hist-3* nonacc) K26a (*hist-3* acc)	5310	none	0	0
6	74-OR14-6A (*ad-5 nic-2*)	1540	nicotinamide	73	4.7
	K26a (*hist-3* acc)	1540	adenine	23	1.5
7	Y152-M43 (*hist-2*) K26a (*hist-3* acc)	10518	none	172	1.7
8	5531A (*pan-1*) C141a (*hist-4*)	7336	none	488	6.7
9	Y152-M108 (*hist-5*) C141a (*hist-4*)	2700	none	693	25.7
10	Y152-M1 (*hist-6*) K34a (*hist-6*)	610	none	0	0
11	Y152-M31 (*hist-7*) Y155-M39 (*ad-4*)	1721	none	161	9.4
12	5.5A-Y175-M275 (*hist-7*) Y155-M39 (*ad-4*)	1217	none	133	10.9
13	Y152-M31 (*hist-7*) 10575a (*tryp-1*)	4341	none	92	2.1
14	Y193-M10 (*hist-7*) 10575a (*tryp-1*)	6410	none	111	1.7
15	Y152-M1 (*hist-6*) 10575a (*tryp-1*)	5610	none	1278	22.8
16	Y152-M105 (*hist-6*) 10575a (*tryp-1*)	3525	none	533	15.1

* Double for conventional map units.

(*pab-1*). More recent crosses (CASE, unpublished) between an *iv-1 inos* double mutant and numerous *hist-1* mutants, which were classified by heterocaryon tests in the experiments discussed here, indicate that these *hist-1* mutants are also clearly located between the *iv-1* and *inos* markers.

hist-2: The *hist-2* locus was shown by HAAS *et al.* (1952) to be in the right arm of linkage group I. The present evidence from crosses no. 1 and 2 between newly acquired *hist-2* mutants and a common *arg-3 nic-2* double indicates that *hist-2* is located between the other two markers and about seven map units to the left of *nic-2*.

hist-3: The *hist-3* locus was shown by HAAS *et al.* (1952) to be in the right arm of linkage group I. Crosses no. 3 and 4 (an *arg-3 nic-2* strain to an accumulating and a nonaccumulating *hist-3* mutant) provide evidence that both *hist-3* mutants are between the other two markers and about three map units to the left of *nic-2*. Cross no. 5 between C140 (*hist-3* by definition) of HAAS *et al.* (1952) and K-26 of MATHIESON and CATCHESIDE (1955) supports the heterocaryon evidence that K-26 is a *hist-3* mutant. Cross no. 6 further confirms this by showing that K-26 is similar to other *hist-3* mutants in crosses to an *ad-5 nic-2* double.

Since *hist-2* mutants and *hist-3* nonaccumulators have indistinguishable accumulation characteristics, it is important to consider whether these two groups are allelic. The first four crosses in Table 5 indicate that the distance between *hist-2* and *nic-2* is about six map units while the *hist-3* to *nic-2* distance is about three units. Cross no. 7 between a *hist-2* and a *hist-3* mutant indicates a map distance between the two loci of 3.2 units. The unpublished evidence of DE SERRES that the *lys-4* locus is situated between *hist-2* and *hist-3* seems to rule out effectively the idea of a single long continuous locus. The evidence indicates that *hist-2* and *hist-3* are linked but nonallelic.

hist-4: The *hist-4* locus has been placed by HAAS *et al.* (1952) in linkage group IV. MITCHELL and MITCHELL (1954) have found it to be located about six units distal to *cot* (colonial-temperature sensitive) in the right arm of linkage group IV. No new mutants were obtained in this group; however, cross no. 8, involving strain C141, shows a *pan-1* to *hist-4* distance of 13.4 map units, which is not inconsistent with the former placement.

hist-5: The *hist-5* group, not previously detected in Neurospora, has similar accumulation characteristics to *hist-4*. This group has not been located genetically. Evidence that the group is different genetically from *hist-4* is found in cross no. 9 which indicates that *hist-4* and *hist-5* exhibit random assortment.

hist-6: A *hist-6* mutant (K-34) was located by MATHIESON and CATCHESIDE (1955) 32.6 units from *pan-1* in linkage group IV. This placement was not further investigated here. Evidence from cross no. 10 confirms the heterocaryon evidence that K-34 is a *hist-6* mutant.

hist-7: The *hist-7* group has been located by crosses no. 11 through 14 in linkage group III about 20 units from *ad-4* and four units from *tryp-1*.

hist-6 and *hist-7* representatives exhibit similar accumulation characteristics;

however, evidence that these are nonallelic is presented in crosses no. 15 and 16, which show that two *hist-6* mutants are clearly not closely linked to *tryp-1* and, therefore, not closely linked to *hist-7*.

The genetic experiments thus confirm the classification derived from heterocaryon complementation tests and provide evidence that, although some of the mutant groups are not distinguishable by tests of accumulants, they are indeed nonallelic.

DISCUSSION

Characterization of mutant groups: The classification of 704 histidine-requiring mutants into seven major groups on the basis of heterocaryon complementation tests has been described. Of the seven groups, four *(hist-1* through *hist-4)* were previously described by HAAS *et al.* (1952) and given group designations by BARRATT *et al.* (1954). An additional group was described by MATHIESON and CATCHESIDE (1955) and is now designated *hist-6*. Of the two new mutant groups, one *(hist-5)* has been tentatively assumed to lack imidazoleacetol phosphate transaminase activity; the other *(hist-7)* has been placed prior to imidazoleglycerol phosphate in the biosynthetic scheme and located in linkage group III. The enzymatic steps between imidazoleglycerol phosphate and histidine reviewed by AMES (1957), i.e., imidazoleglycerol phosphate dehydrase, imidazoleacetol phosphate transaminase, histidinol phosphate phosphatase, and histidinol dehydrogenase, are considered lacking in the mutant groups *hist-1*, *hist-5*, *hist-4*, and *hist-3* accumulators, respectively. Four mutant groups (i.e., *hist-2*, *hist-3* nonaccumulators, *hist-6* and *hist-7*) are placed prior to imidazoleglycerol phosphate in the biosynthetic scheme.

Bratton-Marshall tests were used to detect a diazotizable amine accumulated by representatives of the *hist-6* and *hist-7* groups in growth medium. Evidence has been obtained to indicate that *hist-6* and *hist-7* mutants are blocked later in the biosynthetic scheme than are the *hist-3* nonaccumulators and the *hist-2* mutants.

The genetic experiments have provided evidence that representatives of certain heterocaryon groups which are indistinguishable by accumulation tests are indeed nonallelic. This supports the contention that seven distinct groups are available by ruling out the possibility that any of these pairs of mutant groups should be classified as a single group which exhibits an interallelic complementation pattern.

Absence of hist-4 *mutants:* The striking fact that no *hist-4* mutants have been obtained in these filtration concentration experiments raises the quesion whether *hist-4* might be only one of a number of additional groups of histidine mutants not obtained in these experiments and hence not yet studied. One possible explanation for the absence of *hist-4* mutants is that while C141 (the only *hist-4* mutant) was derived from ultraviolet irradiated conidia, no ultraviolet histidine mutants were screened systematically in the present experiments. On the other hand, AMES (1957) has pointed out that C141, although deficient for histidinol

phosphate phosphatase activity, grows slightly on minimal medium. He suggests that nonspecific phosphatases (inhibited by beryllium ions in the specific assay for histidinol phosphate phosphatase) in the mutant may convert histidinol phosphate to histidinol at a sufficient rate to account for the "leakiness" of C141. If this is a valid suggestion, then all mutants specifically lacking histidinol phosphate phosphatase activity might be expected to be more or less "leaky" and perhaps to grow sufficiently to cause their loss in filtration concentration experiments. *hist-4* mutants may thus, by their very nature, be unobtainable by the filtration concentration method without reflecting unfavorably upon the effectiveness of the method for obtaining a large majority of the mutant classes.

The fact that a substantial number of mutants were screened and no additional groups found suggests that probably all of the histidine mutant groups obtainable by filtration concentration experiments with X-irradiated conidia have been described.

Biochemistry of mutants before imidazoleglycerol phosphate: MOYED and MAGASANIK (1957, 1960) have indicated that the biosynthetic scheme for histidine begins with a reaction between ribose-5-phosphate and adenosine triphosphate to form an intermediate designated by them as compound III. This compound is then split, with the addition of an amino group from glutamine, to yield 5-amine-1-ribosyl-4-imidazolecarboxamide 5′-phosphate and imidazoleglycerol phosphate, the latter of which is converted through four enzymatic steps to histidine. The fact that only two reactions before imidazoleglycerol phosphate have previously been distinguished (with *Salmonella tryphimurium* mutants) and that four nonallelic Neurospora mutant groups are now shown to exhibit blocks before imidazoleglycerol phosphate is striking and may warrant further investigation.

hist-3 *mutants:* The present experiments, utilizing mutants derived from filtration concentration experiments, confirm the evidence of HAAS *et al.* (1952) that the *hist-3* genetic group is heterogenous with respect to physiological characteristics. The *hist-3* mutants may be tentatively separated into accumulators of histidinol and nonaccumulators (accumulating no imidazoles or diazotizable amines). The information from double mutants indicates that nonaccumulators are blocked early in the biosynthetic scheme while histidinol accumulators are blocked later. These findings have led to experiments involving intensive complementation tests and interallelic crosses of *hist-3* mutants. This work will be described in subsequent papers.

SUMMARY

1. Seven hundred and four histidine-requiring Neurospora mutants obtained from filtration concentration experiments have been classified by heterocaryon complementation tests into seven physiological groups. Two groups previously undescribed and one briefly described were assigned the designations *hist-5*, *hist-6*, and *hist-7*. No representatives of the previously described *hist-4* group were found among the mutants studied.

2. The previous subdivision of the *hist-3* groups by HAAS *et al.* (1952) into distinct biochemical classes of mutants has been supported by accumulation

studies with new mutants. Similar evidence has permitted the placement of four classes of mutants before imidazoleglycerol phosphate in the biosynthetic scheme and four after imidazoleglycerol phosphate.

3. Genetic analysis has resulted in the placement of the *hist-7* group in linkage group III. Representatives of certain mutant groups which accumulate similar intermediates have been characterized by genetic tests as nonallelic.

ACKNOWLEDGMENTS

The authors wish to express appreciation for the patient guidance and encouragement of DR. NORMAN H. GILES during these studies and for the advice of DR. NORMA NELSON, DR. C. W. H. PARTRIDGE, and DR. FREDERICK DE SERRES during the preparation of the paper.

LITERATURE CITED

AMES, B. N., 1957 The biosynthesis of histidine; L-histidinol phosphate phosphatase. J. Biol. Chem. **226**: 583–593.

AMES, B. N., and H. K. MITCHELL, 1955 The biosynthesis of histidine; imidazoleglycerol phosphate, imidazoleacetol phosphate, and histidinol phosphate. J. Biol. Chem. **212**: 687–696.

AMES, B. N., H. K. MITCHELL, and M. B. MITCHELL, 1953 Some new naturally occurring imidazoles related to the biosynthesis of histidine. J. Am. Chem. Soc. **75**: 1015–1018.

BARRATT, R. W., D. NEWMEYER, D. D. PERKINS, and L. GARNJOBST, 1954 Map construction in *Neurospora crassa*. Advances in Genet. **6**: 1–93.

BRATTON, A. C., and E. K. MARSHALL, 1939 A new coupling component for sulfanilamide determination. J. Biol. Chem. **128**: 537–550.

CASE, M. E., and N. H. GILES, 1958 Recombination mechanisms at the *pan-2* locus in *Neurospora crassa*. Cold Spring Harbor Symposia Quant. Biol. **23**: 119–134.

DE SERRES, F. J., 1956 Studies with purple adenine mutants in *Neurospora crassa*. I. Structural and functional complexity in the *ad-3* region. Genetics **41**: 668–676.

GILES, N. H., 1955 Forward and back mutation at specific loci in Neurospora. Brookhaven Symposia In Biol. **8**: 103–125.

GILES, N. H., C. W. H. PARTRIDGE, and N. H. NELSON, 1957 The genetic control of adenylosuccinase in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **43**: 305–317.

HAAS, F., M. B. MITCHELL, B. N. AMES, and H. K. MITCHELL, 1952 A series of histidineless mutants of *Neurospora crassa*. Genetics **37**: 217–226.

MATHIESON, M. J., and D. G. CATCHESIDE, 1955 Inhibition of histidine uptake in *Neurospora crassa*. J. Gen. Microbiol. **13**: 72–83.

MITCHELL, M. B., and H. K. MITCHELL, 1954 A partial map of linkage group D in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **40**: 436–440.

MOYED, H. S., and B. MAGASANIK, 1957 The role of purines in histidine biosynthesis. J. Am. Chem. Soc. **79**: 4812–4813.

1960 The biosynthesis of the imidazole ring of histidine. J. Biol. Chem. **235**: 149–153.

NEWMEYER, D., 1954 A plating method for genetic analysis in Neurospora. Genetics **39**: 604–618.

PAULY, H., 1904 Uber die Konstitution des Histidins. Z. Physiol. Chem. **42**: 508–518.

STEWART, R. C., and M. G. SEVAG, 1952 4-Amino-5-imidazolecarboxamide. Role of carbohydrates as critical factor for its accumulation. Arch. Biochem. Biophys. **41**: 9–13.