# NEUROSPORA MUTANTS LACKING ORNITHINE TRANSCARBAMYLASE

V. W. WOODWARD AND PATRICIA SCHWARZ

*Biology Department, Rice University, Houston, Texas*

Received January 10, 1964

SRB and HOROWITZ (1944) suggested that the biosynthesis of citrulline from ornithine involved two reaction steps. Their conclusion was based on the fact that two nonallelic mutants (33442, *arg-2*, and 30300, *arg-3*) gave growth responses when supplied with citrulline or arginine but not with ornithine, and on the assumption that the number of mutations altering a biosynthetic pathway is a measure of the number of steps in the reaction chain.

Subsequent work has vindicated the two-reaction-step hypothesis. JONES, SPECTOR and LIPMAN (1955) have shown that citrulline is synthesized by the coupling of ornithine with carbamyl phosphate (CAP), and that this reaction is catalyzed by the enzyme ornithine transcarbamylase (OTC). From this, one would predict a second step, which if blocked by genetic mutation, would result in a citrullineless phenotype, i.e., the synthesis of CAP, catalyzed by CAP-kinase (CK).

Attempts to coincide the two reaction steps with the two mutants mentioned above were frustrated by the finding that both *arg-2* and *arg-3* possess *in vitro* OTC activity (FINCHAM 1960).

The first clue directed toward solving this problem came when DAVIS (1961) found that extracts of the mutant *s*, a mutation which suppresses certain *pyr-3* mutants (MITCHELL and MITCHELL 1952; DAVIS and WOODWARD 1962) exhibit only 2 to 3 percent as much OTC activity as extracts of wild-type Neurospora. This mutant does not require arginine for growth even though its OTC activity has been much reduced by mutation.

A second important finding has been the association of the *arg-3* mutation with the activity of the enzyme, CAP-kinase (DAVIS 1963). Of the arginine mutants reported to be blocked between ornithine and citrulline, only the function of *arg-2* remains obscure.

The obvious need for additional mutations with altered OTC activity was met with independent searches in DAVIS' laboratory (DAVIS and THWAITES 1963) and in our own (WOODWARD 1963). Two approaches were used by us to obtain OTC-less mutants: the isolation of suppressed *pyr-3* mutants, and the filtration and selective plating technique (WOODWARD, DE ZEEUW and SRB 1954) using *s* as the conidial source.

A genetic and metabolic description of the mutants follows.

## MATERIALS AND METHODS

The new mutations described here reside in the wild-type background of *73a* and *74A*. The

Genetics 49: 845–853 May 1964.

*pyr-3* mutations (SUYAMA, MUNKRES, and WOODWARD 1959; WOODWARD 1962) were isolated in the same background and have been crossed only to mutants of like origin. Mutants with different pedigrees, such as *s*, have been backcrossed to *73a* and *74A* repeatedly. The arginine mutants 33442 and 30300 were used as controls in the OTC assays, and mutants used for the genetical analyses are described in the text.

Growth media have been described (WOODWARD 1962). Growth tests were made in culture tubes in liquid medium and from conidial inocula. Both corn meal and Westergaard and Mitchell's crossing media were used, supplemented with 10 to 30 mg arginine per 100 ml, (SUYAMA, MUNKRES and WOODWARD 1959).

An LD-50 dose of ultraviolet light from a 4w germicidal lamp was used to irradiate the conidia. In all irradiation experiments, a concentration of approximately $10^6$ conidia per ml was irradiated in suspensions of 15 to 30 ml per treatment. Irradiated conidia of the *s* strain were differentially filtered through cheesecloth for 48 to 72 hours, then plated onto agar supplemented with arginine (WOODWARD, DE ZEEUW and SRB 1954) from which suspected mutant colonies were isolated. Following the irradiation of *pyr-3* conidia, 0.25 ml of suspension was spread onto minimal agar medium. The suspected double mutant colonies were isolated from the plates some 48 hours later.

Heterocaryon tests for complementation were carried out as described earlier (WOODWARD 1962).

In preparation for enzyme assays, the mycelia were harvested after 48 hr growth in liquid medium, supplemented with 20 mg arginine per 100 ml, aerated by reciprocal shaking at a temperature of 28°C, washed, and freeze-dried. The dried preparations were powdered in a Wiley mill, then extracted 30 min in .05 M Tris buffer at pH 8.5. The cell debris was removed by centrifugation at 6,000 rpm for 1 hour. The bulk of the nucleic acids were then removed by precipitation with 1/5 volume of 5 percent streptomycin sulfate (JONES 1962) followed by 15 min centrifugation at 6,000 rpm. The resultant supernatant served as the crude extracts for enzyme assays. The specific activity of OTC of each mutant was measured as μg citrulline/mg dry powder/hr, and is expressed as percent of wild-type activity. The quantitative test for OTC activity used in these experiments has been described by DAVIS and WOODWARD (1962).

## RESULTS

*"Suppression" of* pyr-3 *mutants:* Since *s* suppresses only those *pyr-3* mutants exhibiting *in vitro* ATC activity, it was assumed that alleles of *s* would show like specificity of suppression. (Three categories of *pyr-3* mutations have been characterized (WOODWARD 1962): those with no ATC activity and noncomplementary with the other two types; those with no ATC activity and complementary with the third group; i.e., those with ATC activity.) Attempts were made to isolate OTC-less mutants from all three types and, surprisingly, irradiated conidia of the last two types have yielded such mutants.

The double mutants resulting from mutation at the OTC-locus grew into colonies approximately 5 mm in diameter on minimal agar medium. In the absence of some sort of suppression phenomenon such double mutants would require for growth both arginine and pyrimidine. The striking fact, however, was that the suppression effect lasted only a short time; after reaching about 5 mm in diameter the colonies failed to grow, even after transfer to new medium, without an exogenous supply of both arginine and pyrimidine. Suppression has never been effected by reconstituting the double mutants from the single components of the original isolates.

The two strains that have yielded OTC-less mutants have been described in

detail by WOODWARD and DAVIS (1963), namely, $b_2C_7$ and KS-43. The former strain was derived from KS-23, but was changed by mutation to the group displaying ATC activity, suppressibility by *s*, and complementation with KS-43. At least eight OTC-less mutants recovered from irradiated conidia of $b_2C_7$ have been identified (Table 1). One mutant, in like manner, has been isolated from irradiated conidia of KS-43 (Table 1).

*OTC-less mutants derived from* s: In combination with *s* many of the existing arginine mutants show the citrulline-requiring phenotype (MITCHELL and MITCHELL 1952) (Table 2). In particular, the three nonallelic mutants which grow on ornithine, citrulline, or arginine, called *arg-5*, *arg-6*, and *arg-7* grow only on citrulline and arginine when in combination with *s*. Mutants *arg-2* and *arg-3* are changed from leaky citrulline mutants to nonleaky citrulline mutants; several other arginine mutants are altered by *s*. It is evident from these facts that mutants isolated as citrulline requiring types would represent several independent loci.

Irradiated conidia of *s* were selectively filtered and plated onto agar supplemented with citrulline, and colonies from these plates were tested for their

TABLE 1

*New OTC-less mutants*

| Mutant | Origin | Percent of wild-type OTC activity* |
|---|---|---|
| 6–1 | $b_2C_7$ | less than 1% |
| 6–2 | $b_2C_7$ | less than 1% |
| 6–3 | $b_2C_7$ | less than 1% |
| 6–4 | $b_2C_7$ | less than 1% |
| 6–6 | $b_2C_7$ | less than 1% |
| 6–7 | KS–43 | less than 1% |
| 6–8 | $b_2C_7$ | approximately 1% |
| 6–9 | $b_2C_7$ | approximately 1% |
| 7–0 | $b_2C_7$ | less than 1% |
| 103–0 | *s* | less than 1% |
| 102–8 | *s* | less than 1% |

* 2.5 μg dry powder (wild-type) produced 2.6 μg citrulline per hour. 2.5 μg dry powder (*s*) produced 0.02 μg citrulline per hour. The lower limit of sensitivity of the assay procedure is 0.01 μg citrulline per hour.

TABLE 2

*The alteration of phenotype of several arginine mutants by* s

| Mutant | Isolation No. | Phenotype: Without *s* | Phenotype: With *s* |
|---|---|---|---|
| *arg–2* | 33442 = *cit–2* | $cit^-$ leaky | $cit^-$ nonleaky |
| *arg–3* | 30300 = *cit–1* | $cit^-$ leaky | $cit^-$ nonleaky |
| *arg–5* | 27947 = *orn–1* | $orn^-$ | $cit^-$ |
| *arg–6* | 29997 = *orn–2* | $orn^-$ | $cit^-$ |
| *arg–7* | 34105 = *orn–3* | $orn^-$ | $cit^-$ |
| *arg–12* | | $cit^-$ | $cit^-$ |

nutritional requirement. Those isolates with the citrulline phenotype were then crossed to wild type and the progeny were classified according to (a) the presence of ornithine requiring mutants, (b) the presence of leaky citrulline mutants, and (c) the presence of nonleaky citrulline mutants only. The results of many of these crosses can be seen in Table 3.

From the crosses which yielded citrulline mutants only, mutant progeny were tested for OTC activity. The mutants tested showed a range of activity from zero (less than 1 percent of wild-type activity) to 2 or 3 percent of wild-type

TABLE 3

*Results of crossing* s-*arginine double mutants to wild type 73a*

| Complementation group | Unknown double mutant | Progeny from cross to 73a | | | | |
|---|---|---|---|---|---|---|
| | | orn⁻ | cit⁻ leaky | cit⁻ | Wild type | Total |
| A | 100–1 | 0 | 6 | 9 | 10 | 25 |
| C | 100–2 | 0 | 10 | 12 | 27 | 49 |
| A | 100–3 | 0 | 13 | 8 | 28 | 49 |
| A | 100–4 | 0 | 0 | 29 | 22 | 51 |
| D | 100–5 | 18 | 0 | 16 | 30 | 64 |
| B | 100–6 | 0 | 0 | 17 | 19 | 36 |
| B | 100–7 | 0 | 0 | 10 | 53 | 63 |
| B | 100–9 | 0 | 0 | 27 | 23 | 50 |
| E | 101–0 | 0 | 0 | 19 | 30 | 49 |
| B | 101–1 | 0 | 0 | 25 | 34 | 57 |
| A | 101–2 | 0 | 10 | 20 | 22 | 52 |
| A | 101–4 | 0 | 0 | 16 | 17 | 33 |
| D | 101–5 | 10 | 0 | 9 | 27 | 46 |
| B | 101–8 | 0 | 6 | 4 | 53 | 63 |
| A | 102–0 | 0 | 0 | 11 | 24 | 35 |
| A | 102–4 | 0 | 0 | 14 | 20 | 34 |
| A | 102–5 | 0 | 15 | 16 | 29 | 60 |
| A | 102–6 | 0 | 6 | 19 | 34 | 59 |
| A | 102–7 | 0 | 0 | 30 | 26 | 56 |
| H | 102–8 | 0 | 0 | 17 | 51 | 68 |
| G | 103–0 | 0 | 0 | 10 | 21 | 31 |
| — | 103–1 | 10 | 0 | 12 | 45 | 67 |
| A | 103–2 | 0 | 0 | 29 | 28 | 57 |
| I | 103–3 | 17 | 0 | 11 | 26 | 54 |
| A | 103–4 | 0 | 12 | 26 | 22 | 60 |
| B | 103–5 | 0 | 0 | 28 | 33 | 61 |
| F | 103–6 | 0 | 0 | 35 | 27 | 62 |
| A | 103–7 | 0 | 0 | 32 | 34 | 66 |
| A | 103–8 | 0 | 11 | 26 | 29 | 66 |
| A | 103–9 | 0 | 0 | 21 | 43 | 64 |
| A | 104–0 | 0 | 0 | 41 | 24 | 65 |
| B | 104–1 | 0 | 0 | 28 | 30 | 58 |
| A | 104–2 | 0 | 0 | 33 | 34 | 67 |
| B | 104–3 | 0 | 15 | 18 | 29 | 62 |
| J | 104–6 | 3 | .. | 14 | .. | .. |
| B | 104–7 | 0 | 0 | 23 | 29 | 52 |

activity. These mutants are being studied further and will be described in a later paper. Two, of several, mutants with no detectable *in vitro* OTC activity are listed in Table 1, and it can be seen in Table 3 that they belong to different, but overlapping, complementation groups.

The *s*-arginine double mutants were tested in all pairwise combinations for heterocaryon formation. As can be seen from Table 2, at least six independent, citrulline-requiring groups of mutants are expected. The complementation pattern (Figure 1) indicated at least ten different groups, but the ten groups fall into six independent classes. The complementation pattern is not cleancut in that the A and B groups contained both leaky and nonleaky citrulline mutants. The ornithine mutants, however, fall into three groups (D, I, and J), and all of the mutants in the five overlapping groups (B, C, E, G, and H) were citrulline-less. Since all of the mutants subjected to the heterocaryon test were double mutants, it may be unwise to compare the complementation pattern with one like that derived from the *pyr-3* mutants (WOODWARD 1962).

*Genetic location of the OTC locus:* The OTC locus has been named *arg-12* and *s* has been named *arg-12*[s] (DAVIS and THWAITES 1963). *arg-12* is genetically distinct from *arg-2* and *arg-3* (see Tables 4 and 5). It also is evident from Table 4 that *arg-12* is not located in linkage groups I, III, IV, V, VI, or VII.

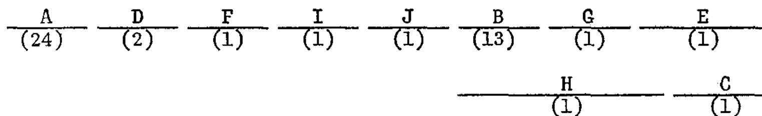


FIGURE 1.—Complementation pattern of 46 *s*-arginine double mutants. The numbers in parentheses refer to the number of mutants in each complementation group (see WOOWARD 1962 for interpretation of complementation maps).

TABLE 4

*Segregation between* arg–12 *and many mutants representing six of the seven linkage groups in Neurospora*

| | | | Progeny | | | |
|---|---|---|---|---|---|---|
| | | | Parental types | | Recombinant types | |
| *a** × *b* | Isolation number | Linkage group | *a* + | + *b* | + + | *a b* |
| *arg–12* × *A/a* | | I L | 49 | 58 | 45 | 76 |
| *arg–12* × *pyr–3* | KS mutants | IV R | 58 | 49 | 45 | 76 |
| *arg–12* × *ylo* | Y30539y | VI L | 24 | 19 | 37 | 22 |
| *arg–12* × *sp* | B132 | V R | 28 | 24 | 30 | 10 |
| *arg–12* × *pyr–2* | 37709 | IV R | 14 | 9 | 10 | 8 |
| *arg–12* × *asp* | C123 | V R | 18 | 24 | 30 | 21 |
| *arg–12* × *ad–8* | Y193–M22 | VI L | 14 | 25 | 21 | 10 |
| *arg–12* × *tryp–1* | 10575 | III R | 13 | 7 | 16 | 8 |
| *arg–12* × *prol–1* | 21863 | III R | 5 | 6 | 10 | 7 |
| *arg–12* × *pan–2* | Y153–M66 | VI R | 10 | 8 | 6 | 11 |
| *arg–12* × *me–7* | 4894 | VII R | 4 | 10 | 15 | 5 |
| *arg–12* × *nic–3* | Y31881 | VII L | 15 | 15 | 7 | 10 |

* The *arg-12* mutants used in these crosses were 6–1, 6–2, 6–3, 6–4, 6–6, 6–9, and 7–0. Many of the crosses represent composite data of two or more *arg–12* mutants.

TABLE 5

*Crosses between* arg–12 *and other* arg⁻ *mutants*

| $a \times b$ | Isolation number | Parentals $a$ + | Parentals + $b$ | Recombinants + + | Recombinants $a$ $b$ | Percent recombination | Percent germination |
|---|---|---|---|---|---|---|---|
| 6–2*a* × *arg–3 A* | ..... | 51* | 37 | 41 | ..* | 64 | 94 |
| 6–3*A* × *arg–1 a* | 46004 | 47* | 34 | 20 | ..* | 40 | 92 |
| 6–3*A* × *arg–8 a* | 44207 | 33* | 16 | 15 | ..* | 47 | 91 |
| 6–2*a* × *arg–9 A* | 35401 | 16* | 9 | 13 | ..* | 68 | 90 |
| 6–2*a* × *arg–2 A* | ..... | 28* | 14 | 14 | ..* | 50 | 95 |
| 6–3*A* × *arg–3 a* | ..... | 42* | 21 | 21 | ..* | 50 | 84 |
| 6–6*a* × *arg–6 A* | ..... | 13* | 16 | 8 | ..* | 43 | 37 |

* Using nutritional tests only, it is impossible to distinguish one parental type from one recombinant type (e.g., $a$ + and $a$ $b$ both require citrulline for growth). Percentage recombination, then, is only an estimate derived by doubling the number of the one recognizable recombinant type and dividing that number by the total number of ascospores tested.

Infertility has made it difficult to analyze progeny from many of the arginine mutants. Where fertility does not pose a problem, some crosses fail to yield recombinant progeny. For example, crosses involving *arg-1* and mutants known to be nonlinked yield no, a few, or as high as 40 percent recombinants (Table 7). Mutants 6–1, 6–3 and 6–4 are known to be linked to markers on linkage group II (Table 6), and they show 50 percent recombination with the mating-type locus, located about one map unit from *arg-1* (Table 4). Many crosses of *arg-1*, *arg-10*, and *arg-12* mutants were completely sterile.

TABLE 6

*Crosses between* arg–12 *and linkage group II markers*

| $a \times b$ | Isolation number | Parentals $a$ + | Parentals + $b$ | Recombinants + + | Recombinants $a$ $b$ | Percent recombination | Percent germination |
|---|---|---|---|---|---|---|---|
| 6–1*A* × *thr–2 a* | 35423 | 37 | 49 | 11 | 10 | 19 | 97 |
| 6–8*a* × *thr–2 A* | ..... | 8 | 8 | 1 | 3 | 20 | 36 |
| 6–9*a* × *thr–2 A* | ..... | 28 | 17 | 4 | 4 | 15 | 96 |
| 7–0*a* × *thr–2 A* | ..... | 21 | 17 | 9 | 4 | 25 | 93 |
| 6–6*a* × *thr–2 A* | ..... | 21 | 26 | 5 | 4 | 16 | 56 |
| 6–3*a* × *thr–3 A* | 44104 | 14 | 28 | 9 | 1 | 18 | 71 |
| 6–6*A* × *thr–3 a* | ..... | 17 | 24 | 8 | 3 | 21 | 70 |
| 7–0*A* × *thr–3 a* | ..... | 15 | 26 | 11 | 2 | 24 | 72 |
| 6–1*A* × *tryp–3 a* | C83 | 15 | 19 | 4 | 9 | 27 | 87 |
| 6–3*A* × *tryp–3 a* | ..... | 19 | 18 | 8 | 7 | 28 | 94 |
| 6–4*A* × *tryp–3 a* | ..... | 14 | 19 | 7 | 4 | 25 | 88 |
| 6–9*a* × *tryp–3 A* | ..... | 19 | 15 | 6 | 7 | 27 | 94 |
| 6–9*a* × *arg–5 A* | ..... | 130* | 90 | 18 | ..* | 15.1 | 79 |
| 7–0*a* × *arg–5 A* | ..... | 117* | 90 | 23 | ..* | 20.0 | 77 |
| 6–6*a* × *arg–5 A* | ..... | 147* | 150 | 22 | ..* | 13.7 | 80 |
| *s A* × *arg–5 a* | ..... | ..† | 160 | 198† | 29 | 15.0 | 75 |

* $a$ + and $a$ $b$ are indistinguishable since both require citrulline for growth.
† $a$ + ($s$ +) and + + (wild type) cannot be distinguished by nutritional tests, but + $b$ (*arg–5*) and $a$ $b$ ($s$ *arg–5*) can be distinguished since + $b$ responds to ornithine and $a$ $b$ does not.

TABLE 7

*A sample of crosses involving* arg–1 *as one parent*

| | Parentals | | Recombinants | | | |
|---|---|---|---|---|---|---|
| *a* × *b* | *a* + | + *b* | + + | *a b* | Percent recombination | Percent germination |
| 6–1*A* × *arg–1 a* | 52 | 53 | 0 | . . | 0 | 83 |
| *arg–1a* × *arg–5 A* | 24 | 22 | 0 | . . | 0 | 77 |
| 6–4*A* × *arg–1 a* | 29 | 39 | 7 | . . | 18.6 | 92 |
| 6–3*A* × *arg–1 a* | 47 | 34 | 20 | . . | 40 | 92 |

Crosses of many *arg-12* mutants and *s*, to markers distributed throughout the genome point to the location of both *s* and *arg-12* on linkage group II. Since crosses between *arg-12* mutants are infertile, it is difficult to prove allelism among them. However, the nonleaky *arg-12* mutants have been tested for complementation and shown to be allelic by that criterion, i.e., no mutants classified here as *arg-12* have shown full complementation with other *arg-12* mutants. Also, the *arg-12* mutants show linkage to *thr-2* (16 to 25 percent recombination), to *thr-3* (18 to 24 percent recombination), to *tryp-3* (25 to 28 percent recombination), and to *arg-5* (13.7 to 20 percent recombination). Difficulties in locating a more precise position arise from the absence of suitable markers in the relevant region of linkage group II.

MITCHELL and MITCHELL (1952) obtained some evidence that *s* is linked to *arg-5*, but the paucity of progeny observed made it difficult to establish the fact conclusively. Coupled with the present data, however, the case is strong for both the allelism of *s* and *arg-12*, and for the location of *arg-12* near *arg-5* on linkage group II.

## DISCUSSION

Many attempts have been made to isolate arginine-requiring mutants, and many thousands of such mutants have been isolated. The repeated failures to isolate OTC-less mutants have been difficult to interpret, but it now seems that the sudden loss of their ability to suppress *pyr-3* mutants affords a clue. Presumably a conidium in which a mutation at the *arg-12* locus occurs must contain either a large quantity of free arginine, which is unlikely since other arginine mutants behave differently, or sufficient OTC to supply enough arginine for substantial increase. Such a quantity of OTC would permit sufficient mycelial growth to preclude the mutants from being isolated by the filtration-selective plating procedure. DAVIS and THWAITES (1963) did isolate one *arg-12* mutant from wild-type conidia by the filtration technic, but it seems unlikely that the method enhances the recovery of OTC-less mutants.

The *s* conidia, on the other hand, are known to possess only about 3 percent as much OTC as the wild type. Conidia of the *s* mutant ought to be a more fruitful source of *arg-12* mutants, then, simply because the newly induced mutant conidium would be unable to germinate owing to the reduction of OTC. The facts are consistent with the hypothesis.

It is known that certain *pyr-3* mutants are relieved of their pyrimidine requirement in combination with *s*, but the addition of arginine offsets the suppression effect. The role of arginine in this regard is not clear, but it is clear that the *pyr-3s* double mutant synthesizes sufficient arginine for growth, whereas the *pyr-3 arg-12* (No. 6–1, for example) double mutant does not. As long as the latter double mutant synthesizes arginine via residual OTC it does not require pyrimidine, but as soon as an exogenous supply of arginine is required, pyrimidine must be supplied as well. The study of some of the mutants with intermediate levels of OTC may shed more information on this problem.

Much of this work was done in the laboratory of PROFESSOR D. G. CATCHESIDE, at the University of Birmingham. His hospitality and stimulation are acknowledged with gratitude. The research was supported by Research Grants RG-8653 and GM 10206–01 from the National Institutes of Health and G.20376 from the National Science Foundation. The assistance of WILDA COOK is much appreciated.

## SUMMARY

Mutants lacking ornithine transcarbamylase, the enzyme which couples ornithine to carbamyl phosphate, have been isolated by two methods: (1) as suppressors of the class of *pyr-3* mutants displaying *in vitro* aspartic transcarbamylase activity, and (2) by the filtration and selective plating technic, using conidia of a suppressor mutation, *s*; this strain suppresses, among other mutants, the class of *pyr-3* mutants referred to above. The enzymatic phenotypes of these mutants are described and, although infertility makes it difficult to prove allelism, genetic analyses indicate that all of the mutants studied are located near *arg-5* on linkage group II.

## LITERATURE CITED

DAVIS, R. H., 1961 Suppressor of pyrimidine-3 mutants of Neurospora and its relation to arginine synthesis. Science **134**: 470–471. —— 1962 A mutant form of ornithine transcarbamylase found in a strain of Neurospora carrying a pyrimidine-proline suppressor gene. Arch. Biochem. Biophys. **97**: 185–191. —— 1963 Neurospora mutant lacking an arginine-specific carbamyl phosphokinase. Science **142**: 1652–1654.

DAVIS, R. H., and V. W. WOODWARD, 1962 The relationship of gene suppression and aspartate transcarbamylase activity in *pyr-3* mutants of Neurospora. Genetics **47**: 1075–1083.

DAVIS, R. H., and W. M. THWAITES, 1963 Structural gene for ornithine transcarbamylase in Neurospora. Genetics **48**: 1551–1558.

FINCHAM, J. R. S., 1960 Genetically controlled differences in enzyme activity. Advances in Enzymol. **22**: 1–43.

JONES, M. E., 1962 Carbamyl phosphate synthesis and utilization. Methods in Enzymology **5**: 903–925.

JONES, M. E., L. SPECTOR, and F. LIPMANN, 1955 Carbamyl phosphate, the carbamyl donor in enzymatic citrulline synthesis. J. Am. Chem. Soc. **77**: 819–820.

MITCHELL, M. B., and H. K. MITCHELL, 1952 Observations on the behavior of suppressors in Neurospora. Proc. Natl. Acad. Sci. U.S. **38**: 205–214.

SRB, A. M., and N. H. HOROWITZ, 1944 The ornithine cycle in Neurospora and its genetic control. J. Biol. Chem. **154**: 129–139.

SUYAMA, Y., K. D. MUNKRES, and V. W. WOODWARD, 1959 Genetic analyses of the *pyr-3* locus of *Neurospora crassa.* Genetica **30**: 293–311.

WOODWARD, V. W., 1962 Complementation and recombination among *pyr-3* heteroalleles of *Neurospora crassa.* Proc. Natl. Acad. Sci. U.S. **48**: 348–356. —— 1963 Ornithine transcarbamylaseless mutants of Neurospora. (Abstr.) Proc. 11th Intern. Congr. Genet. **1**: 51.

WOODWARD, V. W., J. R. DEZEEUW, and A. M. SRB, 1954 The separation and isolation of particular biochemical mutants of Neurospora by differential germination of conidia followed by filtration and selective plating. Proc. Natl. Acad. Sci. U.S. **40**: 192–200.

WOODWARD, V. W., and R. H. DAVIS, 1963 Co-ordinate changes in complementation, suppression and enzyme phenotypes of a *pyr-3* mutant of *Neurospora crassa.* Heredity **18**: 21–25.