STERLING EMERSON
California Institute of Technology

Chapter 12

Biochemical Models of Heterosis in Neurospora

Some of the things that have been learned about gene controlled reactions in Neurospora can be used in forming a picture of how individual genes contribute to heterosis. I wish to consider especially those examples which indicate that heterozygosity at a single locus may influence the growth of an organism to a considerable extent.

It should be noted at the beginning, however, that one is not justified in assuming that the situations found in Neurospora are necessarily similar to those occurring in the higher organisms in which heterosis is ordinarily studied. It may be unwise to assume that any two organisms are essentially similar. There are special reasons for caution in making comparisons between Neurospora and higher plants and animals, since the nuclear and chromosomal basis for the expression of heterosis is so dissimilar. On the other hand, there is a considerable accumulation of information about the parts played in the physiology and biochemistry of Neurospora by individual genes (Beadle, 1948; Horowitz, 1950) and, with proper caution, we may assume that some of this information may have rather broad application.

In any haploid organism, such as the ascomycetous fungus Neurospora, in which there is a single set of genes in each nucleus, such phenomena as dominance, heterozygosis, and heterosis cannot occur. There is, however, a condition known as heterocaryosis which permits a loose approximation to each.

CHARACTERISTICS OF HETEROCARYONS

The plant body of Neurospora can be said to be made up of cells, but they are very different from the cells of higher plants. In the first place, the cells contain a large and variable number of nuclei in a common cytoplasm. The so-called cells themselves are not as discrete as cells are generally supposed

to be. The walls between them have perforations which permit both cytoplasm and nuclei to move from cell to cell. If all nuclei are identical, their movement and distribution is probably of minor importance, but if they are not identical there may be effects of considerable consequence arising from irregularities in nuclear distribution.

There are two ways in which a mixture of different kinds of nuclei within a single cell may come about. In the growth resulting from a sexually produced ascospore, or from a uninucleate asexual microconidium, all nuclei are directly descended from a single haploid nucleus. Barring mutation, they should all have the same genetic constitution. After the growth has become

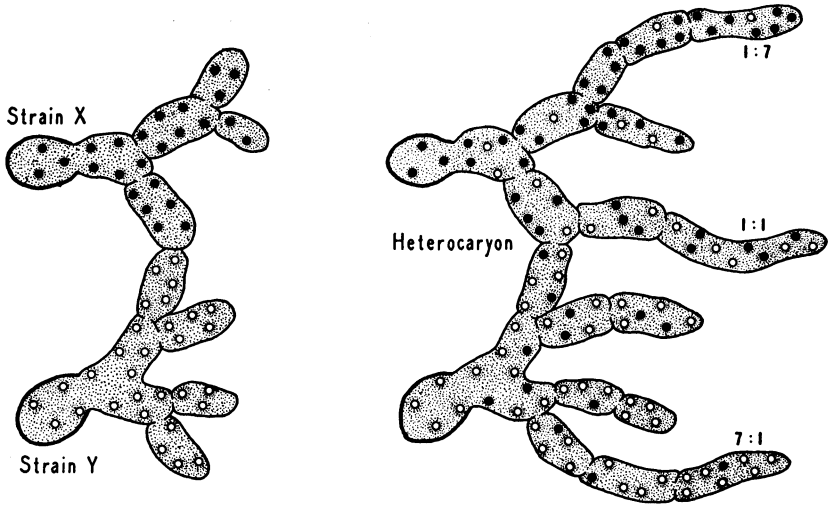


FIG. 12.1—Heterocaryon formation resulting from hyphal fusion (a diagram).

multinucleate, if a mutation should occur in one nucleus, the descendants of that nucleus would then have a different genetic constitution from the remaining nuclei in the common cytoplasm, and a condition of heterocaryosis would exist. The second way in which heterocaryons arise is from the direct fusion of branches or hyphae of different strains, with the subsequent intermingling of their nuclei. By the latter method, heterocaryons of predetermined genetic constitution can be made at will.

The controlled production of heterocaryons is shown diagrammatically in Figure 12.1. Strain X is represented as having black nuclei to distinguish them from the nuclei of strain Y, which are pictured as being white. After fusion between hyphae, nuclei of strain Y may migrate into cells of strain X, and those of X into Y. It is possible that different hyphal tips, growing from this common mass of cells, will have different relative numbers of the two sorts of nuclei, as illustrated by the ratios 1:7, 1:1, and 7:1 in three of

the hyphal tips. To prove that two kinds of nuclei were present in the same cells of such heterocaryons, Beadle and Coonradt (1944) cut off single hyphal tips, transferred them to fresh medium, and then identified two kinds of nuclei in the resulting growth by genetic test.

Where there is freely branching filamentous growth, as in Neurospora, it is possible for the two types of nuclei in a heterocaryon to become sorted out

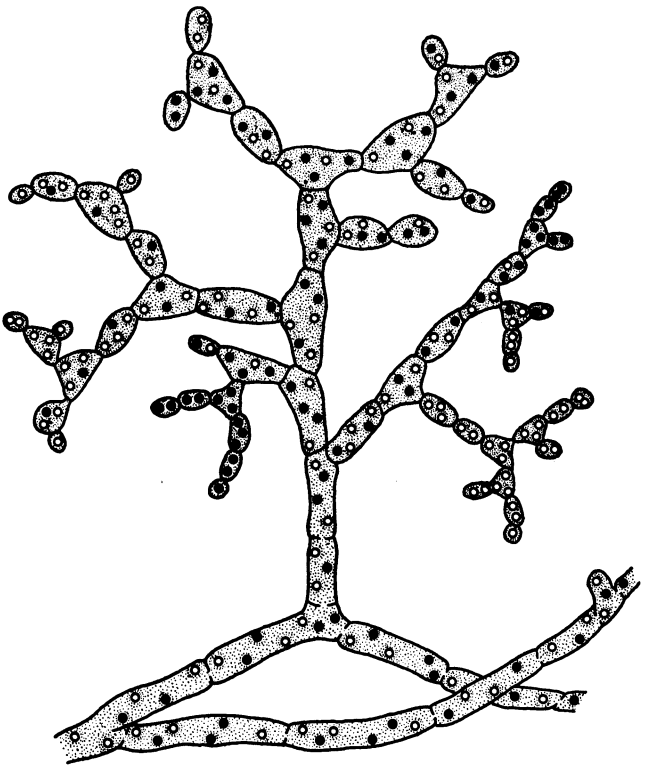


FIG. 12.2—Somatic segregation of dissimilar nuclei in the formation of conidia (a diagram).

purely as a matter of chance, as illustrated in a schematic way in Figure 12.2. This diagram actually represents an erect fruiting branch, or conidiophore, on which the asexual spores are born. The conidia of Neurospora have variable numbers of nuclei, but generally more than one. Dodge (1942) proved that two kinds of nuclei were present in the same cell of a heterocaryon by growing cultures from single conidia, and then showing by genetic test that some of these cultures had both types of nuclei. In some instances he was able to distinguish the heterocaryotic and both homocaryotic types in culture derived from single conidia by their morphological characteristics.

The essential differences between Neurospora and higher organisms with

respect to heterosis result from the points just noted. In a diploid which is heterozygous for a single gene pair, both alleles are present in the same nucleus and in equal dosage. Whereas in the corresponding haploid heterocaryon, the two alleles are present in different nuclei, and the relative proportions of the two alleles vary with the frequencies of the two types of nuclei. All cells of a diploid heterozygote have the same genetic constitution, but there can be a considerable variation in genetic constitution in different parts of a heterocaryotic individual. Interactions between alleles, by which I mean such things as the expression of dominance, must result from the ability of genes to act at some distance in heterocaryons, in which there is no possibility of an intimate association of alleles within a nucleus (Lewis, 1950). It is considerations such as these that show that dominance and heterosis-like effects in Neurospora are only approximations to the phenomena as known in diploid organisms.

HETEROSIS IN HETEROCARYONS

An enhancement of growth, closely simulating heterosis, in heterocaryons of *Neurospora tetrasperma* was reported by Dodge in 1942. In this paper he distinguished between heterocaryotic vigor and the hybrid vigor of diploid organisms along much the same lines as I have just done. He suggested that the heterocaryotic vigor observed might be the result of complementing growth factors whose production was controlled by the two types of nuclei (Robbins, 1950). It was later (Dodge, Schmitt, and Appel, 1945) demonstrated that genes responsible for enhanced growth segregated and recombined in a normal fashion. These studies showed that genes residing in different nuclei, but in a common cytoplasm, can cooperate in establishing conditions favoring rapid growth, and that a condition resembling hybrid vigor occurs.

Meantime, Beadle and Coonradt (1944) had reported on heterocaryons between pairs of mutant strains of *Neurospora crassa*, each of which is unable to synthesize a particular vitamin or amino acid. Each mutant strain by itself is unable to grow unless supplied with its specific growth requirement, but nine heterocaryons involving different combinations of seven mutant strains grew at rates approximating that of wild type without the addition of growth factors. The authors conclude that the wild type allele is dominant to the mutant allele in each of the examples studied.

Beadle and Coonradt note further that in such heterocaryons, in which there is the opportunity for great diversity in the relative numbers of the two types of nuclei in different hyphal tips, those tips having the most favorable proportions of nuclei should grow most rapidly. Conversely, rapidly growing hyphae should have the two sorts of nuclei in roughly optimal proportions. In heterocaryons involving pairs of mutant strains, Beadle and Coonradt found nuclear ratios varying between approximately 1:1 and almost 20:1. They interpreted these results to mean that the wild type alleles

of different mutant genes have different degrees of dominance. A strongly dominant wild type allele will need to be present in relatively few nuclei—say one in twenty.

A heterocaryon between two mutant strains could grow at the maximum rate over a large range of nuclear proportions, provided the wild type alleles concerned were both strongly dominant. A weakly dominant wild type allele, on the other hand, must be present in a large proportion of the nuclei—say nineteen of twenty—to ensure vigorous growth. Heterocaryons in which the wild type alleles concerned are both weakly dominant could never result in vigorous growth, since the two wild type alleles cannot both be present in excess, one being in one type of nucleus and the other in the remaining nuclei.

HETEROSIS DUE TO HETEROZYGOSITY AT ONE LOCUS

The heterosis effect in heterocaryons studied by Beadle and Coonradt results from the mutually complementary nature of the nuclei involved. For each deleterious mutant allele in one nucleus there is the corresponding favorable and dominant wild type allele in another. In contrast to these there are other heterocaryons (briefly reported in Emerson, 1947) in which the nuclei differ in only one gene, yet which still show the heterosis effect. Heterocaryons in which some nuclei carry the dominant allele and some the recessive are superior to homocaryons, all of whose nuclei have the dominant allele, or all the recessive.

Heterocaryotic Suppression of the Sulfonamide-requiring Character

Most of the heterocaryons of this sort that have been found so far have involved the so-called sulfonamide-requiring mutant strain. At 35° on minimal medium, this strain makes extremely poor growth, but it does keep creeping along. After varying lengths of time, it frequently happens that the growth will change to a rapid vigorous type. Growth curves of six cultures which have reverted to something approaching wild type growth are shown in Figure 12.3. When the mycelium had reached the end of the growth tubes, inocula from the newest growth were introduced into fresh tubes containing minimal medium, resulting in the growth curves shown in the upper part of the figure.

From these curves it can be seen that the reverted type of growth usually persists through a conidial transfer. After the mycelium had reached the end of the second tube, conidia were removed and used in outcrosses to wild type to determine the genetic constitution of their nuclei. These tests showed that each of the six cultures represented in Figure 12.3 was a heterocaryon. One type of nucleus present in each heterocaryon was identical to those in the original sulfonamide-requiring strain. The second type of nucleus in each also carried the sulfonamide-requiring gene, *sfo* (in one instance, that derived from culture number 1 in Figure 12.3, the *sfo* gene itself was somewhat

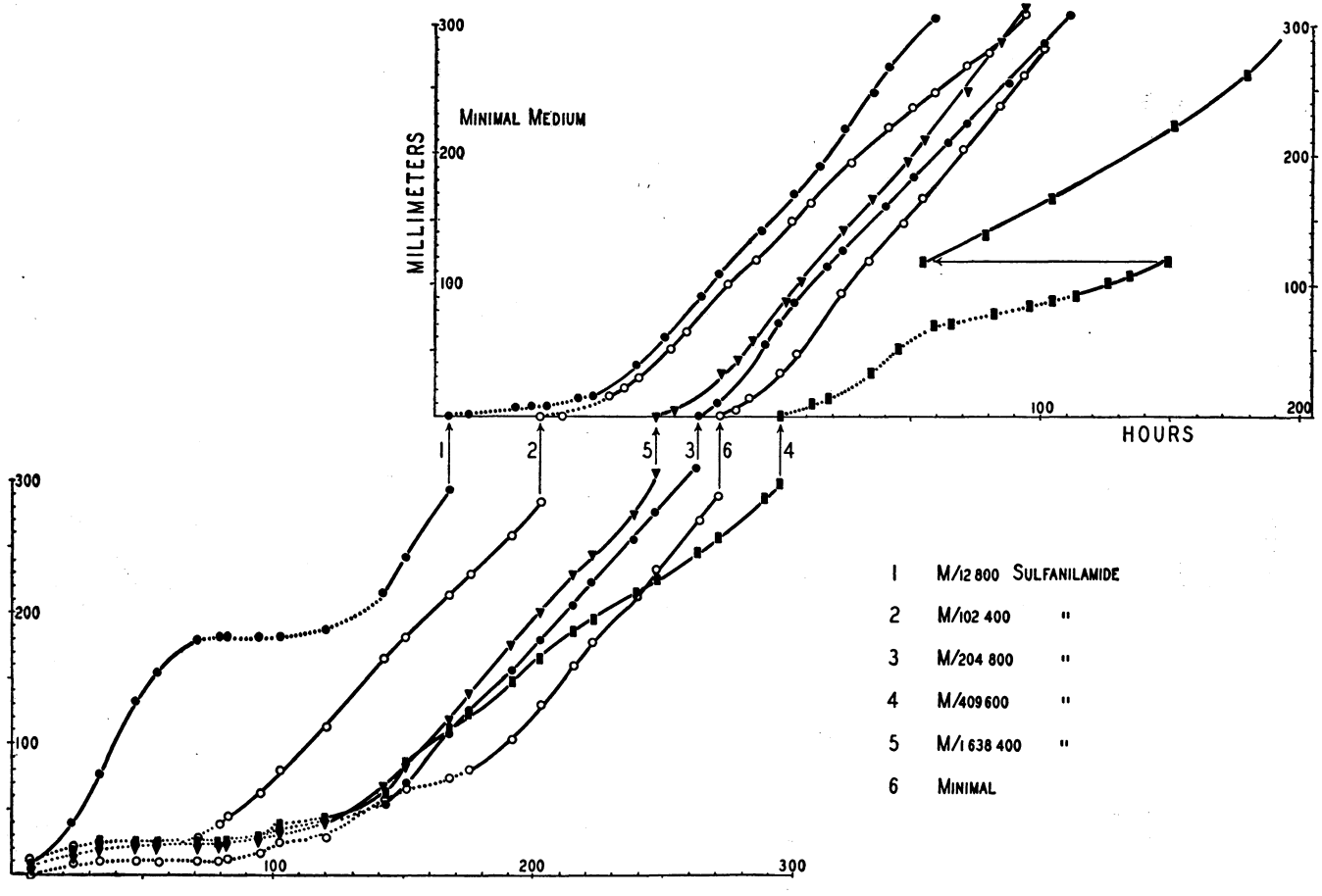


FIG. 12.3—Growth curves of the sulfonamide-requiring strain of Neurospora showing the development of *reverted* type of growth. Solid lines represent vigorous growth; dotted lines, poor growth. Cultures grown by the tube method (Ryan, Beadle, and Tatum, 1943) at 36.4°.

modified), and in addition a second mutant gene, *S*, which was presumably responsible for the change in growth (Table 12.1).

The new mutants appearing in the heterocaryons have been called suppressors because they overcome the deleterious effect of the sulfonamide-requiring gene in heterocaryons. Actually they are not like the usual suppressors, because in homocaryotic strains which also carry the sulfonamide-requiring gene they do not result in wild type growth.

Growth characteristics of strains homocaryotic for four of these suppressors, with and without the sulfonamide-requiring gene, are represented in

TABLE 12.1

DISTRIBUTION OF NUCLEI IN THE HETEROCARYONS REPRESENTED IN FIGURE 12.3

From Culture Tube Number	Nuclei	
	sfo, +	*sfo*, *S*
1	3	5
2	6	5
3	15	2
4	8	1
5	8	1
6	1	14

Figure 12.4. From these growth curves it can be seen that wild type (+, +) is neither inhibited by sulfanilamide in a concentration of 2×10^{-4} *M*, nor stimulated by *p*-aminobenzoic acid in a concentration of 10^{-4} *M* when grown at 35°, and is only slightly inhibited by sulfanilamide at 25°. At 35° growth of the sulfonamide-requiring strain (*sfo*, +) is stimulated by sulfanilamide and inhibited by *p*-aminobenzoic acid, though neither substance has an appreciable effect at 25° in the concentrations used.

The suppressor from tube 1 (+, *S*-1) does not grow at 35°, and grows slowly on all media at 25°. The suppressor from tube 2 (+, *S*-2) differs from wild type principally in taking longer to attain its maximum growth rate, though there is also some stimulation by sulfanilamide at 35°. When combined as a double mutant with the sulfonamide-requiring gene (*sfo*, *S*-2), it almost approximates the growth of wild type. The suppressor from tube 4 (+, *S*-4) differs from wild type in being stimulated by *p*-aminobenzoic acid and inhibited by sulfanilamide, the inhibition being stronger at 25°. In combination with the sulfonamide-requiring gene (*sfo*, *S*-4) it resembles the sulfonamide-requiring strain itself except that there is a long lag phase on sulfanilamide at 35°, and inhibition at 25°. The suppressor from tube 6, either alone

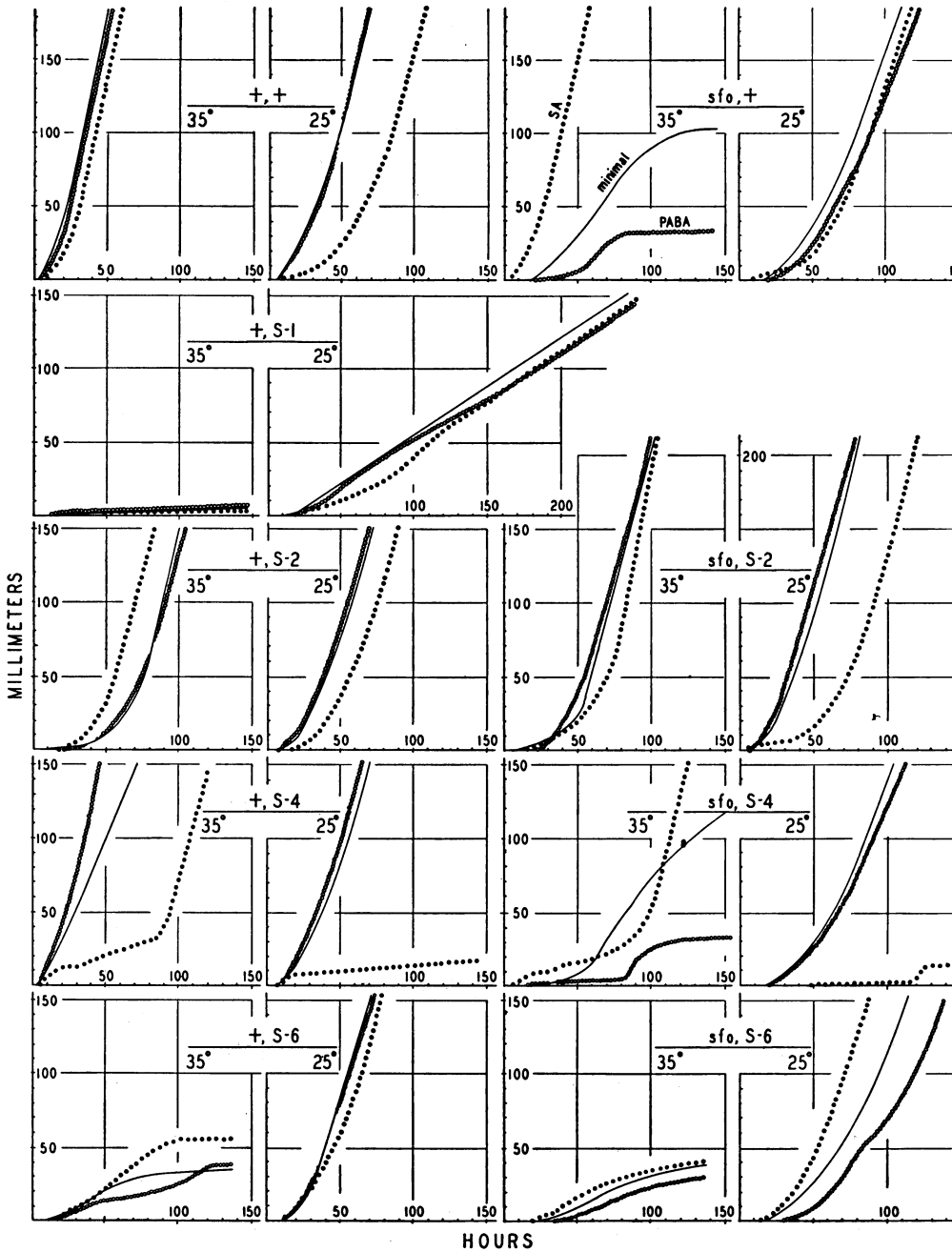


FIG. 12.4—Growth curves of suppressor strains in absence and presence of the sulfonamide-requiring gene (at 35° and 25° on minimal medium (light line), 10^{-4} *M* *p*-aminobenzoic acid (line of open circles —PABA), 2×10^{-4} M sulfanilamide (dotted line —SA).

(+, *S*-6) or in combination with the sulfonamide-requiring gene (*sfo*, *S*-6), grows very poorly at 35°.

Of those illustrated, suppressors numbered 4 and 6 are perhaps the most significant to the present discussion. When combined with the sulfonamide-requiring gene (*sfo*, *S*-4 and *sfo*, *S*-6), neither grows well on minimal medium at 35°. Yet heterocaryons between either of these double mutants and the sulfonamide-requiring strain are enabled to grow quite well under those conditions. In these heterocaryons the sulfonamide-requiring gene is present in all nuclei, in some of which it is combined with a suppressor. The suppressor is not capable of overcoming the ill effects of the sulfonamide-requiring gene when present in all nuclei, but is effective when present in only some of them.

Biochemical Basis for the Sulfonamide-requiring Character

This seeming paradox becomes less important once the nature of the reaction controlled by the sulfonamide-requiring gene is understood (Zalokar, 1948, 1950; Emerson, 1950). The diagrams in Figure 12.5 illustrate some of the important reactions involved. There are a large number of amino acids, vitamins, components of nucleic acid, and so on, that are essential to growth. But we shall consider only two amino acids, methionine and threonine, and the vitamin *p*-aminobenzoic acid. Para-aminobenzoic acid is involved in a number of reactions essential to growth, one of which is the final step in the synthesis of methionine from homocysteine. Wild type carries out all essential reactions and produces all essential growth factors, with the exception of biotin which must be supplied to all strains.

The reaction governed by the sulfonamide-requiring gene has not yet been identified, but we know quite a little about it. It requires the presence of both homocysteine and *p*-aminobenzoic acid. Presumably homocysteine is used as a substrate in this reaction, and *p*-aminobenzoic acid, or a derivative, is needed as a catalyst. The reaction either results in the destruction of threonine or else interferes with its normal utilization, so that the sulfonamide-requiring strain has too little threonine for growth. We also know that more homocysteine is required for this deleterious reaction than for the synthesis of methionine, and that in the presence of limiting amounts of homocysteine, the synthesis of methionine goes on without any interference with the utilization of threonine.

Furthermore, the deleterious reaction requires larger amounts of *p*-aminobenzoic acid than are needed for all essential reactions combined. Only about half as much is needed in the synthesis of methionine, about a quarter as much in the production of purines, and very much less still for other essential, but still unidentified factors. Both wild type and the sulfonamide-requiring strain produce about one hundred times as much *p*-aminobenzoic acid as is needed for all essential reactions.

We know of three ways in which the deleterious reaction leading to threo-

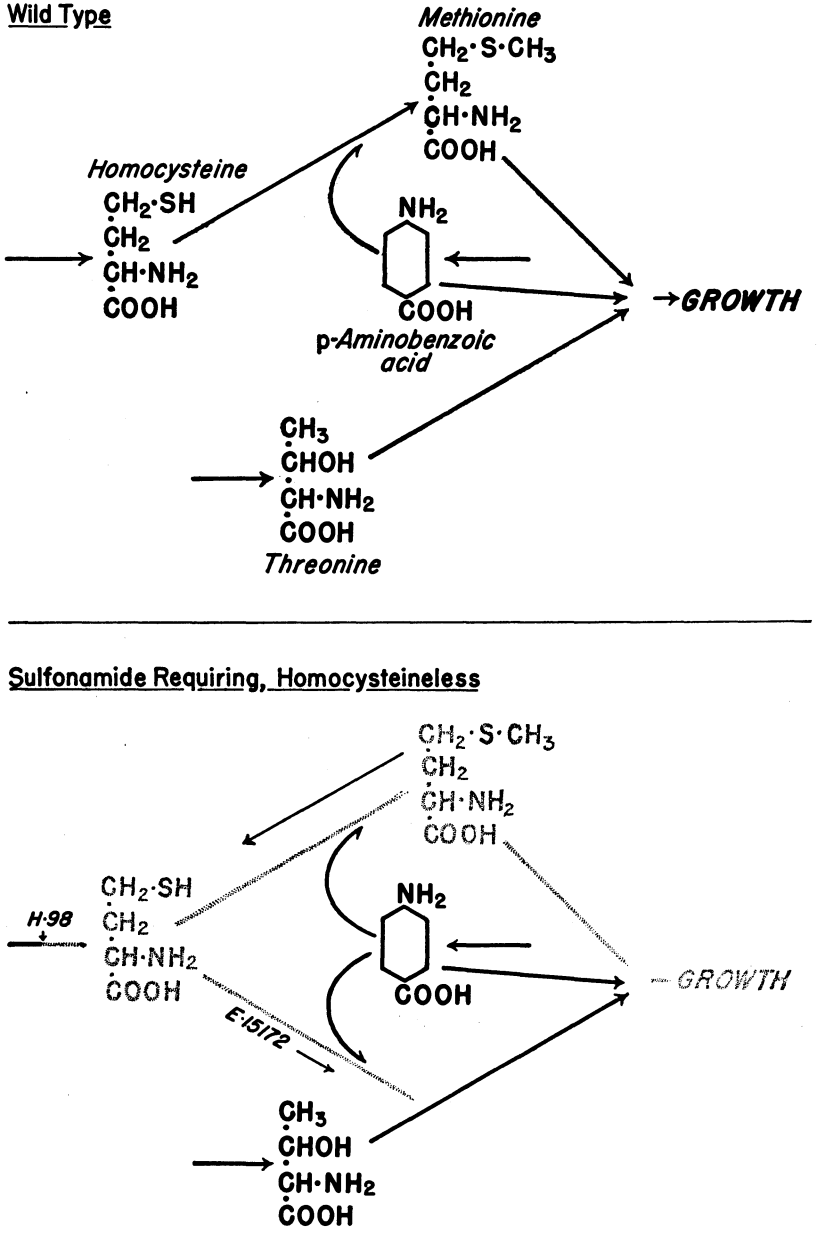
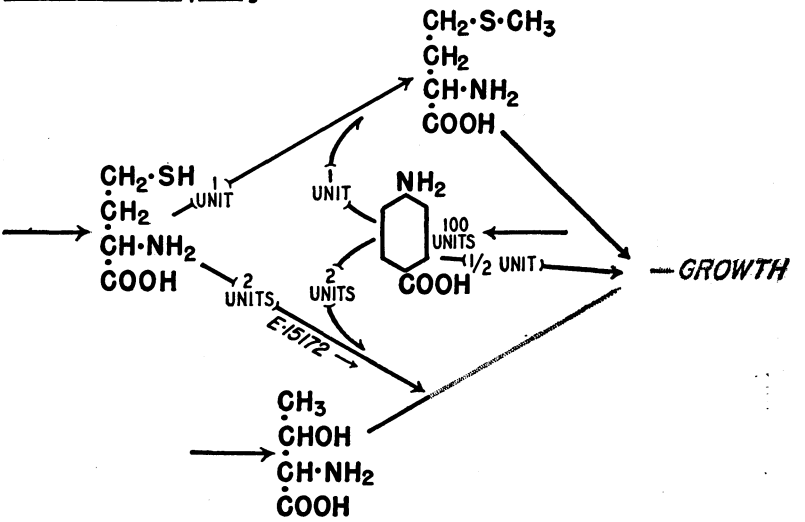


FIG. 12.5—Certain biochemical reactions essential to growth in Neurospora as influenced by genes of the sulfonamide-requiring strain (E-15172), the homocysteineless strain (H-98), and the aminobenzoicless strain (1633).

Sulfonamide Requiring



Sulfonamide Requiring, Aminobenzoicless

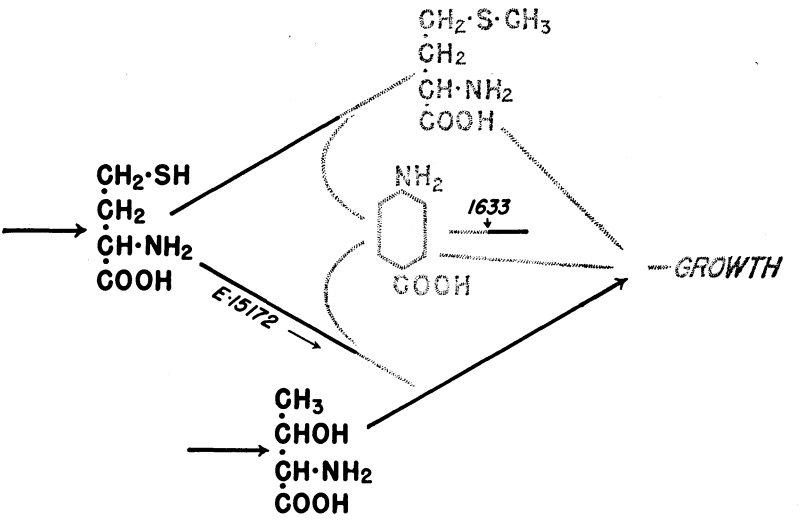


FIG. 12.5—*Continued*

nine deficiency can be prevented by genetic means. The simplest is of course by introducing the wild type allele of the sulfonamide-requiring gene, but the other two are of more interest. One of these is by introducing a genetic block to the synthesis of homocysteine. Mutant strain H-98 blocks the terminal step in the synthesis of homocysteine. In the double mutant—sulfonamide-requiring, homocysteineless—there is no interference with the availability of threonine for growth, since the deleterious reaction does not take place in the absence of homocysteine. In the absence of homocysteine, however, there can be no synthesis of methionine, so that the double mutant fails to grow because of a methionine deficiency. The double mutant will grow if supplied with exactly the right amount of methionine—more inhibits growth, because methionine is degraded to homocysteine which then supports the deleterious reaction (Zalokar, 1950).

The remaining method is to introduce a genetic block to the synthesis of *p*-aminobenzoic acid. In the double mutant—sulfonamide-requiring, aminobenzoicless—there is again no interference with the utilization of threonine since there is no *p*-aminobenzoic acid to catalyse the deleterious reaction. There is again a deficiency for methionine, because *p*-aminobenzoic acid is needed in its synthesis. There is also a deficiency of *p*-aminobenzoic acid for other essential processes. The double mutant will grow if supplied just the right amount of *p*-aminobenzoic acid to satisfy the essential requirements, but not enough to stimulate the deleterious reaction (Zalokar, 1948).

Model Heterocaryons

It can be seen that the simple sulfonamide-requiring mutant on the one hand, and the two double mutants on the other, have different deficiencies. One produces methionine and *p*-aminobenzoic acid, but not enough threonine. The others produce sufficient threonine, but no methionine, and in one case, no *p*-aminobenzoic acid. In heterocaryons between the simple and double mutants, the two types of nuclei should complement each other in the production of essential growth substances. If the nuclear ratios can be so adjusted that the different substances are produced in appropriate amounts, vigorous growth should result. Heterocaryons involving the simple sulfonamide-requiring mutant and the double mutant sulfonamide-requiring, aminobenzoicless have resulted in vigorous growth (Emerson, 1948) in every test so far made. Growth curves of some of these heterocaryons are illustrated in Figure 12.6.

Growth of these heterocaryons is usually not maintained at a constant rate. Growth may stop completely after a time, or it may nearly stop and then start again. This is believed to be due to fluctuations in the ratio of the two kinds of nuclei in the advancing hyphal tips. Apparently there must be many times as many double mutant nuclei as simple sulfonamide-requiring nuclei to result in a favorable combination. This is not surprising since the

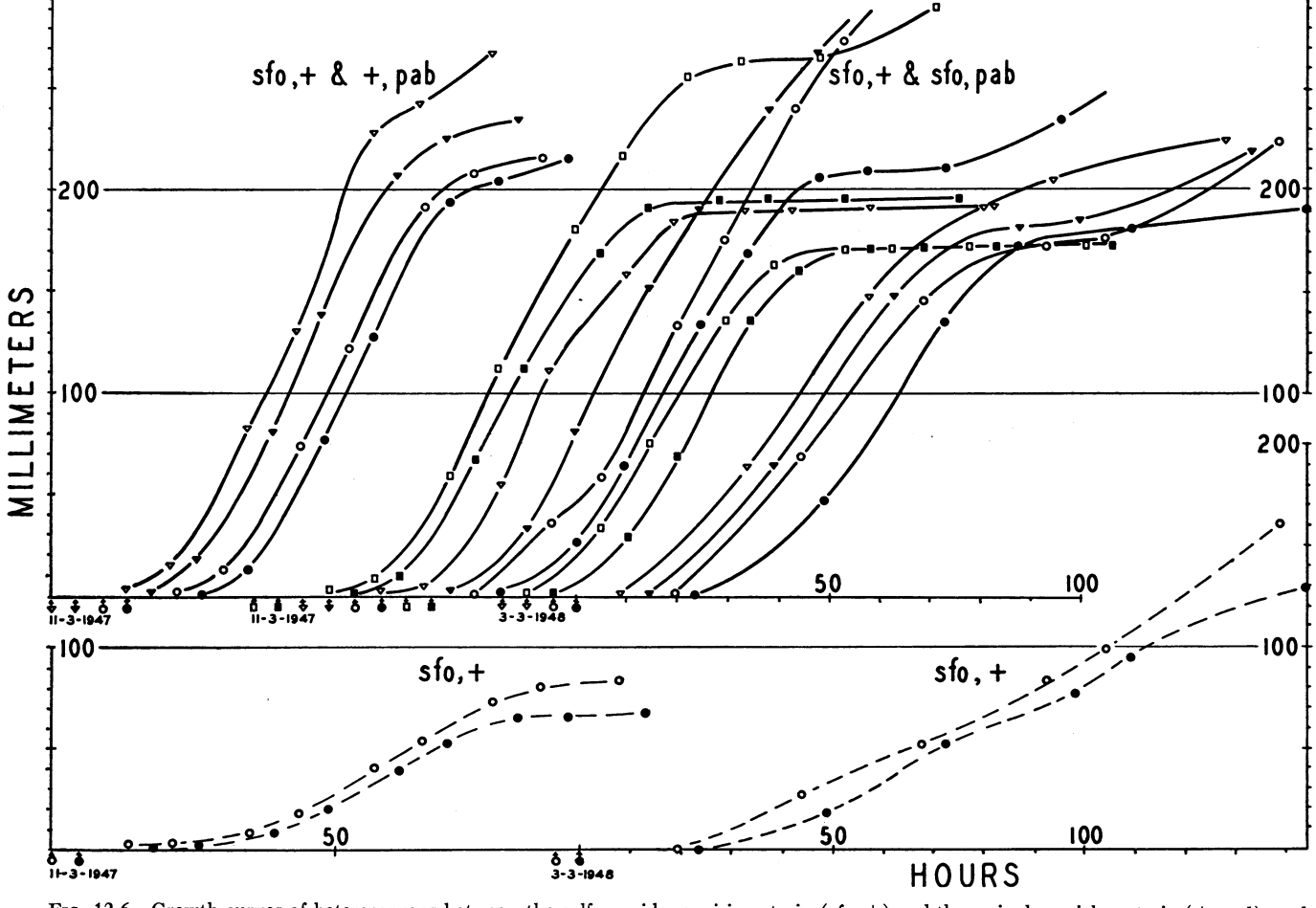


FIG. 12.6—Growth curves of heterocaryons between the sulfonamide-requiring strain (*sfo*, +) and the aminobenzoicless strain (+, *pab*), and between the sulfonamide-requiring strain and the double mutant (*sfo*, *pab*). Growth curves of the sulfonamide-requiring strain by itself are shown at the bottom of the figure, the aminobenzoicless and double mutant controls grew not at all. All cultures on minimal medium at 35°.

sulfonamide-requiring strain produces something in the order of one hundred times as much *p*-aminobenzoic acid as is required for essential reactions, or about fifty times as much as is required for the reaction which makes threonine unavailable for growth.

Limited direct tests of nuclear frequencies in such heterocaryons indicate that nuclei carrying only the sulfonamide-requiring gene are much less frequent than those carrying the aminobenzoicless gene as well. In one test of about one hundred nuclei, all proved to be double mutants. In another test, conidia from heterocaryons were transferred to fresh growth tubes which contained a concentration of sulfanilamide sufficient to inhibit growth of the double mutant very strongly and still be favorable to the growth of the simple sulfonamide-requiring mutant. Only one of five such transfers grew—again suggesting that simple sulfonamide-requiring nuclei were infrequent.

If in order to have rapid growth there must be many double mutant nuclei and few simple mutants, it is not surprising that vigorous growth should cease rather suddenly. Ryan, Beadle, and Tatum (1943) have shown that growth substances can be transported for a distance of about one centimeter in the mycelium of Neurospora. One sulfonamide-requiring nucleus at a distance of about a centimeter from the tip might supply enough *p*-aminobenzoic acid for the growth of that tip. But as the tip grows, that nucleus might easily be left behind. A deficiency of *p*-aminobenzoic acid would then develop in the tip, and growth would be arrested unless a nucleus of the proper constitution happened to migrate into the tip.

Attempts to obtain rapidly growing heterocaryons involving the sulfonamide-requiring mutant and the sulfonamide-requiring, homocysteineless double mutant were unsuccessful. It may be that it is impossible to have a nuclear ratio which will produce sufficient, but not too much methionine, and at the same time sufficient threonine for the requirement of the heterocaryon.

Interpreting Suppressor Heterocaryosis Based on Model Experiments

The heterocaryons between the sulfonamide-requiring mutant and its double mutants with aminobenzoicless and homocysteineless were set up as models which should duplicate the behavior observed in the sulfonamide-requiring strain when suppressor mutations occurred, provided the interpretation placed on them was correct. For this purpose, the results obtained were gratifying. We should like to know just where each of the suppressor mutations studied fits into the biochemical scheme, but at present it can be shown only that they fit in a general way.

Four suppressors in the first lot of six (those illustrated in Fig. 12.4), which are the only ones that have been studied in any detail at all, apparently represent mutation at four different loci, though almost no direct tests

for allelism are available. The inference that they are distinct genes is based on the data summarized in Table 12.2.

The reactions controlled by the suppressor genes have not been identified. Suppressor *S*-4 is stimulated in growth by additional *p*-aminobenzoic acid, and is inhibited considerably by sulfanilamide at concentrations twenty times less than that required to inhibit wild type. It is possible that a deficient amount of *p*-aminobenzoic acid is produced by this mutant, which would make it approximate the condition in one of the model heterocaryons. Growth of suppressor *S*-2 is somewhat stimulated by sulfanilamide (Fig. 12.4) and by threonine, in this respect resembling the sulfonamide-requiring mutant which it "suppresses." It is even more stimulated by the purine,

TABLE 12.2

EVIDENCE SUGGESTING THAT SUPPRESSORS S_1, S_2, S_4, AND S_6 ARE DIFFERENT GENES

Suppressor	Second Division Segregation	Relation to 1633	Genetically Independent of
S_1	25%	none	.....
S_2	50%	allele ?	.....
S_4	0%	none	S_6
S_6	60%	none	S_4

adenine, as shown by the growth curves in Figure 12.7. It was previously known that in the presence of methionine, adenine reduces the normal requirement for *p*-aminobenzoic acid to about one-tenth its usual value. This suggested that the production of adenine also requires *p*-aminobenzoic acid. The reaction controlled by this suppressor may thus be closely related to that controlled by the sulfonamide-requiring gene. No clues have turned up to indicate how the reactions governed by the remaining suppressor mutations may be related to these.

In the living cell of Neurospora the reactions which are influenced in one way or another by the amount of available *p*-aminobenzoic acid must be fairly numerous. The production of adenine and methionine requires the presence of this vitamin as does the reaction in the sulfonamide-requiring mutant which makes threonine unavailable.

Strauss (1950) has studied a mutant strain (44602) which requires pyridoxine unless grown at high pH with ammonia as nitrogen source. He found that under the latter conditions it is inhibited by methionine, and that this inhibition is reversed by sulfanilamide, as if *p*-aminobenzoic acid were required for the inhibition. Still another interrelationship has been found by Shen (1950) in studies of a mutant strain (84605) which requires sulfur in a

form at least as reduced as thiosulfate. At 35° it has no other requirement, but at 25° it needs reduced sulfur, generally supplied as the amino acid cysteine, and also tyrosine. When methionine is supplied as the source of sulfur at 25°, growth is strongly inhibited by choline. Under these conditions, choline does not inhibit at 35°, but there is an unexpected stimulation in growth by *p*-aminobenzoic acid at that temperature.

Mutant strains have been reported on two occasions which require either choline or *p*-aminobenzoic acid—choline may be the source of the methyl

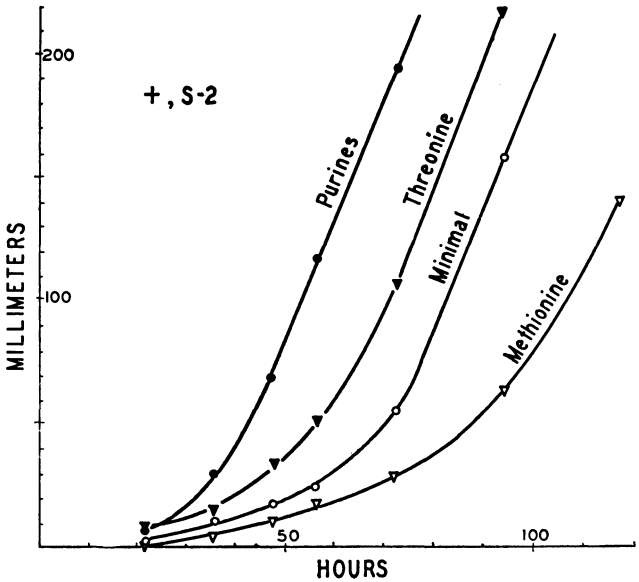


FIG. 12.7—Growth curves of suppressor mutant strain S-2 on minimal medium, on threonine (5 mg/100 ml), on methionine, and on purines (5 mg/100 ml each adenine sulfate and guanine hydrochloride) at 35°.

group of methionine. Strehler (1950) has reported a strain which requires either methionine or *p*-aminobenzoic acid. There is also a suggestion that *p*-aminobenzoic acid may be involved in the metabolism of lysine. In Neurospora this is suggested only because the double mutants between the sulfonamide-requiring strain and two different mutants which are unable to synthesize lysine do not grow on any combination of growth factors we have tried. In bacteria a strain has been found which requires either lysine or *p*-aminobenzoic acid as a growth factor (Koft *et al.*, 1950), strengthening the supposition of a similar interrelationship in Neurospora.

These observations are referred to at this time because they indicate that there are a large number of metabolic reactions that are in one way or another related to the availability of *p*-aminobenzoic acid. These reactions must themselves be interrelated in the sense that an upset in one of them

may have a strong effect on one or more of the others, possibly through changing the availability of *p*-aminobenzoic acid or a derivative. The model heterocaryon experiments described earlier show that it is possible for one mutation to cause an upset in one reaction and thus be detrimental to growth, and for a second mutation to restore conditions favorable to growth by actually interfering with a different reaction which is itself essential to growth, but which is interrelated with the first reaction. In the reactions related to the metabolism of *p*-aminobenzoic acid, there is sufficient complexity to account for the occurrence of a large number of different suppressors of the sulfonamide-requiring character.

DISCUSSION

It has been shown that increased vigor can result from heterocaryosis in which the two kinds of nuclei differ by only one pair of alleles. This may be true only under very special conditions such as have been present in the examples discussed. On the other hand, it is possible that the necessary conditions may be met with rather frequently in Neurospora, as suggested by the following examples.

In mutant strains which have specific requirements for particular amino acids, it is commonly found that their growth is inhibited by the presence of other amino acids which do not ordinarily interfere with growth. Some mutants which require an outside source of threonine are strongly inhibited by methionine, (Teas, Horowitz, and Fling, 1948). Mutants specifically requiring lysine are inhibited by arginine (Doermann, 1944), and so on. In each of these instances, the inhibition by a particular amino acid is competitively antagonized by the specific amino acid required by the strain in question. The growth of these mutants should be favored by a reduction in the amount of the inhibiting amino acid, as would occur if some of the nuclei carried a genetic block to its synthesis.

In extreme cases, the specific requirement for an amino acid may not result from a failure in its synthesis, but from an oversensitivity to the inhibiting amino acid. Thus, the sulfonamide-requiring strain can be said to be oversensitive to homocysteine in a way that leads to a requirement for threonine. One of the lysineless mutants (33933) seems to be oversensitive to arginine in much the same way. Heterocaryons having the lysineless gene in all nuclei, some of which also carry a genetic block to the synthesis of arginine (from strain 36703), make considerable growth on minimal medium, whereas neither the lysineless nor the double mutant does (Fig. 12.8).

Mary B. Mitchell (personal communication) recently observed that the stock cultures of certain lysineless mutants (4545, 15069, and 33933) had become less sensitive to inhibition by arginine. Tests of these showed that they were heterocaryons, some of whose nuclei were unchanged. Some carried mutant genes which lowered the sensitivity to arginine inhibition while

leaving the requirement for lysine. These heterocaryons were more vigorous than the original lysineless strain, but no more vigorous than the pure double mutant strains extracted from the heterocaryons.

In studies on reverse mutation in a leucineless strain (33757), Ryan and Lederberg (1946) found that heterocaryons, whose nuclei differed only in

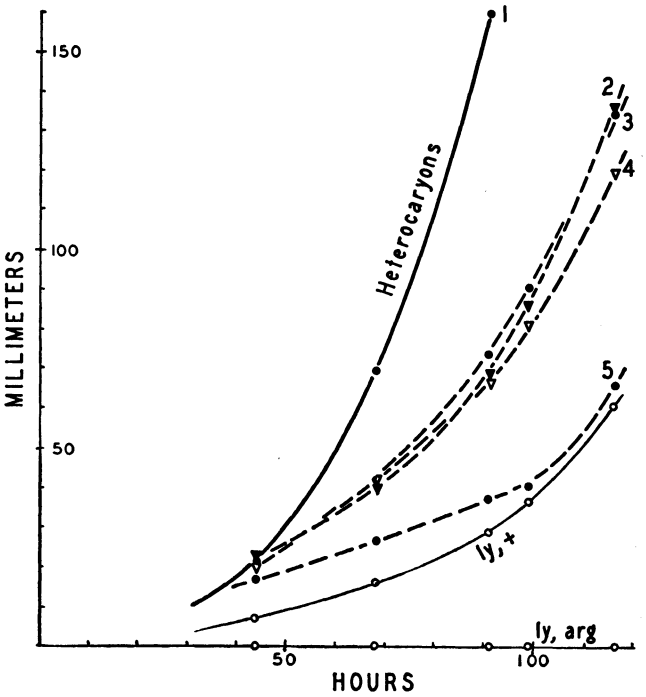


FIG. 12.8—Growth curves of heterocaryons between lysineless (*ly*, +) and lysineless, argininelessness (*ly*, *arg*) strains of Neurospora at 35° on minimal medium. Curve 1: heterocaryon in which both nuclear types were of mating type A; curves 2 to 5: heterocaryons made up of nuclei of different mating types (*ly*, +, *A* and *ly*, *arg*, *a*)—*cf.* Beadle and Coonradt (1944).

that some carried the wild type allele and some the mutant allele of the leucineless gene, almost invariably reverted to the homocaryotic condition. By the time growth had reached the end of a tube containing minimal medium, nothing but wild type nuclei remained. In tubes containing limiting concentrations of leucine, nothing but leucineless nuclei were present after a short period of growth. This was under conditions where the growth rate of the leucineless strain is considerably less than that of wild type. Under both of these conditions, the heterocaryon is at a strong disadvantage compared to its components. It is not known whether or not there is a particular concentration of leucine which would favor the heterocaryon.

Houlahan and Mitchell (1948) have studied the interactions of mutant strains involved in the metabolism of pyrimidines and lysine. A pyrimidineless mutant (37301) has a specific requirement for pyrimidine. There is a suppressor of this mutant which enables it to grow without added pyrimidine, unless arginine is also added, whereupon the pyrimidine requirement is restored. One lysineless strain (33933) can utilize α-amino adipic acid in place of lysine. As a double mutant with the pyrimidine suppressor, it can still use α-amino adipic acid, but requires four times as much as the simple lysineless strain unless small amounts of arginine, or an arginine precursor, are added. The double mutant combining this lysineless with the pyrimidineless mutant is unable to use α-amino adipic acid unless a small amount of lysine is added—arginine is ineffective in this instance. A second lysineless mutant (4545), which has a specific requirement for lysine and which secretes pyrimidines into the medium, behaves in a predictable fashion as a double mutant with pyrimidineless, or its suppressor, but not as the triple mutant lysineless, pyrimidineless, suppressor of pyrimidineless. Instead of requiring only lysine for growth, this triple mutant also requires pyrimidines and arginine. This example is cited as another in which metabolic interactions may be as complex as in those discussed earlier which depend in one way or another on *p*-aminobenzoic acid.

Applicability to Classical Heterosis

Observations relating to one-gene heterosis in higher plants are discussed in other papers in this series (Crow, Hull, Jones, and Whaley). Studies of Neurospora heterocaryons have shown that a very similar phenomenon occurs under certain special physiological conditions. In a particular genetic background, the amount of an essential metabolite normally produced has deleterious consequences which are removed by reducing the dosage of a gene responsible for the production of that metabolite. This reduction was brought about through heterocaryosis in the studies reported, but it should also result from heterozygosis under similar physiological conditions. There is nothing in the studies of heterocaryosis in Neurospora to suggest that one-gene heterosis is of general occurrence and importance, or that other examples should have similar biochemical backgrounds.