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*EVIDENCE FOR AN INTERRELATION IN THE METABOLISM OF LYSINE, ARGININE AND PYRIMIDINES IN NEUROSPORA**

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The behavior of a strain of *Neurospora crassa* carrying the pyrimidineless gene *3a* (37301) and *s*, a second mutant gene which suppresses the effect of *3a*, has been described briefly in an earlier publication.[1] A strain carrying *3a* alone has an absolute requirement for pyrimidine, while *3a-s* reaches about one-half the maximum growth of the wild type on the unsupplemented basal medium.[2] However, if arginine is present in this medium the pyrimidine requirement is again manifested. The effect of arginine may be suppressed in turn by the addition of lysine. This response to arginine and lysine suggested the possibility that one or both of these amino acids are involved in the biosynthesis of pyrimidines. More recent investigations have provided evidence that a relationship does exist, but its nature is not yet understood.

Of the two lines of evidence which indicate this interrelation perhaps the most convincing is the fact that two of four non-allelic lysineless mutants[3] accumulate pyrimidine compounds in their culture media. One of the compounds has been isolated and identified as uracil. A second substance, more active biologically, has been shown to be present but has not been isolated. There is some evidence which suggests that this compound is uridine.

A second line of evidence is based on growth responses of multiple mutant strains derived from crosses of lysineless mutants to pyrimidineless *3a-s*. The unexpected behavior of several of these strains has not been satisfactorily interpreted, but, in the opinion of the authors, such an interpretation must involve an interrelation in the metabolism of pyrimidines and lysine, and, possibly of arginine as well.

Accumulation of Pyrimidines by Lysineless Strains.—Four lysineless

mutants, shown by Doermann[3] to be non-allelic, have been tested. The isolation numbers are 33933, 37101, 4545 and 15069; for simplicity they will be called *ly 1*, *2*, *3* and *4*. After the finding by Borsook, *et al.*,[4] that lysine is converted to α amino adipic acid by mammalian tissue slices and homogenates it was shown that this amino acid can be used as a substitute for lysine by strain *ly 1* but not by *ly 2*, *3* and *4*.[5] From this it is inferred that the reaction blocked in strain *ly 1* precedes those blocked in the other strains.

Accumulated pyrimidine compounds have been demonstrated in culture fluid from *ly 3* and *4* but not in that from *ly 1* and *2*. The amount found, in terms of cytidine sulfate activity for the pyrimidineless strain *3a*, varied from 0.1 to 0.2 mg. in 10 ml. of culture fluid. The substances promote growth of three non-allelic pyrimidine-requiring strains, 37301 or *3a*, 263 and 38502.[6] The accumulation was observed in four-day cultures containing 1 mg. of L(+) lysine monohydrochloride in 20 ml. of medium. This quantity of lysine is sufficient to allow half-maximum growth of the lysineless strains. When higher lysine concentrations were used little or no pyrimidine activity was detected.

Of the four lysineless strains, *1* and *3* have been more thoroughly tested. In repeated experiments *ly 3* has not failed to show the accumulation under the conditions given above while *ly 1* has always failed to do so. Moreover, no accumulation by the double mutant *ly 1–ly 3* has been observed. In three experiments with *ly 2* and *ly 4* the former has behaved like *ly 1* and the latter like *ly 3*. Culture fluid from wild type (Abbott 4) and from an adenine-requiring strain 27663-44206 supplied a limiting quantity of adenine, showed no biological activity for the pyrimidineless strains.

Uracil was isolated from a culture of *ly 3* grown four days at 25°C. in a five-gallon bottle containing 15 l. of basal medium supplemented with 750 mg. of L(+) lysine monohydrochloride. The material was first concentrated by adsorption on Norite (2 mg. per ml. of culture fluid) and elution with boiling 10% aniline solution. The active compounds were then precipitated by the addition of excess silver nitrate at about pH 8. Silver was removed as the sulfide, the volume reduced by evaporation and crystalline compound was obtained. After two recrystallizations from hot water the compound melted with decomposition at 335°C. (corr.). Literature values for uracil are 338°C., 335°C. The ultra-violet absorption spectrum was identical to that of uracil and the biological activity for *pyr 3a* was also the same.

It seems clear that all of the biological activity found is not due to uracil. The activity of a number of partially purified preparations exceeded by a considerable margin that of the quantity of uracil which will remain dissolved in the same volume of water. Moreover, solutions of such highly active preparations showed the maximum absorption of ultra-violet light at the same wave-length (259 mμ) in 0.1 *N* NaOH as in 0.1 *N* HCL. This

is characteristic of the spectrum of uridine, while solutions of uracil, on the other hand, show a change from 259 mμ in acid to 283 mμ in alkali.

It was found that a small quantity of cytidine, which has the same biological activity as uridine (about 30 times that of uracil[6]) will greatly enhance the activity of uracil. A mixture of 0.1 mg. of cytidine sulfate and 1.6 mg. of uracil is equivalent in activity to 8 mg. of uracil alone. It seems safe to conclude, therefore, that a pyrimidine derivative with the activity of uridine or cytidine is also accumulated. The ultra-violet absorption spectra mentioned above indicate that it is uridine.

TABLE 1

REQUIREMENTS FOR HALF-MAXIMUM GROWTH OF SINGLE AND MULTIPLE MUTANT STRAINS

STRAIN	REQUIREMENT—MICROMOLS/20 ML. MEDIUM
s	0
pyr 3a	cytidine sulfate 1.6; uracil 54.0
	cytidine sulfate 0.31 + uracil 2.7
pyr 3a-s	0
ly 1	L—lysine 5.5
	DL α amino adipic acid 6.2
ly 1-s	L—lysine 5.5
	DL α amino adipic acid 25.0
	DL α amino adipic acid 6.2 + L—arginine 0.47
	DL α amino adipic acid 6.2 + DL citrulline 5.7
	DL α amino adipic acid 6.2 + DL ornithine 12.0
ly 1-pyr 3a	L—lysine 5.5 + cytidine sulfate 1.6
	DL α amino adipic acid 6.2 + cystidine sulfate 1.6 + L—lysine 0.55
ly 1-pyr 3a-s	L—lysine 5.5
ly 3	L—lysine 5.5
ly 3-s	L—lysine 5.5
ly 3-pyr 3a	L—lysine 5.5 + cytidine sulfate 1.6
ly 3-pyr 3a-s	L—lysine 5.5 + cytidine sulfate 1.6 + L—arginine 2.4

Double and Triple Mutants Involving Ly 1; Pyr 3a and s.—The requirement for lysine due to the mutant gene *ly 1* is not affected by the presence, in the same strain, of *s* or *pyr 3a*, nor is the pyrimidine requirement or *pyr 3a* altered by the presence of *ly 1*. Growth of the triple mutant *ly 1–pyr 3a-s*, when supplied adequate lysine, parallels that of *pyr 3a-s* on the unsupplemented basal medium. However, with respect to their ability to use α amino adipic acid as a substitute for lysine each of the three strains, *ly 1-s*, *ly 1-pyr 3a* and *ly 1-pyr 3a-s* differs from strain *ly 1*.

The quantity of α amino adipic acid required to initiate growth of strain *ly 1-s* is about four times that which is necessary for half-maximum growth of *ly 1*, but if arginine, citrulline or ornithine is added then the response of this strain is very nearly like that of the single mutant. Citrulline is less effective than arginine and ornithine is still less effective; also the effect of the latter is rather erratic. The quantities required are given in table 1.

This response to these amino acids is of particular interest because of their effect upon the lysineless strains and upon *pyr 3a-s*. Their presence in the culture medium inhibits the utilization of lysine by all of the lysineless strains[7] and prevents growth of strain *pyr 3a-s* in the absence of pyrimidine.[1] The relative effectiveness of arginine, citrulline and ornithine is the same in the three cases.

Strain *ly 1-pyr 3a* does not grow when supplied α amino adipic acid with cytidine and cannot be made to do so by the addition of arginine, citrulline or ornithine. However, if a small quantity of lysine is added, about one-tenth the requirement for half-maximum growth, then, in the presence of adequate cytidine, the response of this strain to α amino adipic acid is like that of *ly 1*. Utilization of this compound by *ly 1-s* is apparently not affected by lysine. When supplied lysine in addition to quantities of α amino adipic acid smaller than that required to initiate growth, this strain responds as it does to the same quantity of lysine alone.

Utilization of α amino adipic acid by *ly 1-pyr 3a-s* has not been demonstrated although the following additions have been tried: lysine; arginine plus cytidine; and lysine plus arginine plus cytidine.

No inhibitory effects have been observed of cytidine upon the utilization of α amino adipic acid by strain *ly 1*, nor of this amino acid upon the utilization of cytidine by *pyr 3a*. Growth of strain *pyr 3a-s* is not affected by α amino adipic acid.

Double and Triple Mutants Involving Ly 3, Pyr 3a and s.—Requirements for lysine and cytidine of the double mutants *ly 3-s* and *ly 3-pyr 3a*, like the corresponding combinations with *ly 1*, have not been found to differ from those of the single mutants. Strain *ly 3-pyr 3a-s*, on the other hand, departs markedly from the behavior which would be predicted for it on the basis of characteristics of the single and double mutants involving these three genes. It does not grow when supplied lysine alone, but requires pyrimidine and arginine as well. Moreover, in order to allow maximum growth, lysine and arginine must be supplied in a molar ratio of 2.3 to 1. The requirement for pyrimidine is not surprising since the suppressor does not function if arginine is present unless the lysine concentration is about six times that of arginine. When lysine and arginine are supplied in suitable concentrations then the response of this strain to cytidine is the same as that of *pyr 3a*. Table 1 lists the requirements for half-maximum growth of the various single and multiple mutant strains.

There are two points of similarity between *ly 3-pyr 3a-s* and the argininess mutant 36703. First, neither citrulline nor ornithine will satisfy the arginine requirement.[8] Second, both strains are inhibited by lysine if the molar ratio of lysine concentration to arginine concentration is 3.5 to 1 or greater. For this reason strains of the constitutions *arg-s* and *arg-pyr 3a* were considered of possible interest and were prepared. However, no

departures from predicted behavior have been observed. *Arg-s* responds to arginine and lysine as does *arg* alone, while requirements of *arg-pyr 3a* for arginine and cytidine are like those of *arg* and *pyr 3a*. Combinations involving *arg* and *ly 1* or *ly 3* may prove of interest but they have not yet been obtained.

Verification of Genetic Constitution of Multiple Mutant Strains.—The various double and triple mutants were obtained from crosses of *pyr 3a-s* to *ly 1*, *ly 3* and *arg*. *Ly 1-s*, *ly 3-s* and *arg-s* were selected from asci containing four spores carrying *pyr 3a* alone. The other four spores would, of course, carry *s* along with the third mutant gene involved in the cross. The double mutants of *pyr 3a* with *ly 1*, *ly 3* or *arg* were obtained from asci which gave rise to four wild-type appearing strains and four having the appropriate double requirement, lysine plus pyrimidine or arginine plus pyrimidine. *Ly 1-pyr 3a-s* came from an ascus from which were obtained four wild-type appearing strains and four requiring only lysine. The ascus from which *ly 3-pyr 3a-s* was derived gave rise to four wild-type strains and four which failed to grow when supplied lysine plus cytidine. The identity of these two strains was further checked by recovering from crosses to wild-type strains carrying *pyr 3a-s* and others requiring lysine alone or lysine plus pyrimidine. *Arg-pyr 3a* had, of course, to be crossed to wild type in order to distinguish it from *arg-pyr 3a-s*.

In the case of each multiple mutant described, isolates from several different asci were tested and found to have the same characteristics.

Discussion.—The fact that obstruction of the biosynthesis of lysine at either of two points results in accumulation of pyrimidines, while interruption of the series at two other points does not produce this result, establishes an interdependence in the metabolism of pyrimidine and lysine in Neurospora. Such a relationship is also strongly indicated by the fact that introduction of a genetic block in the series of reactions by which the pyrimidine ring is synthesized interferes with the utilization of a precursor of lysine, α amino adipic acid, by strain *ly 1-pyr 3a*. The behavior of *ly 1-s* and *ly 1-pyr 3a-s*, as well as that of *ly 1-pyr 3a*, suggests that utilization of this compound is dependent upon the *pyr 3a* reaction.

No satisfactory scheme has been devised which will explain how lysine metabolism is related to pyrimidine biosynthesis and in what way arginine is involved.

Summary.—Of four non-allelic lysineless strains two have been shown to accumulate pyrimidines, while two do not. One of the accumulated compounds has been isolated and identified as uracil. Another has been tentatively identified as uridine.

The double and triple mutant combinations of the pyrimidineless gene, *3a* and its suppressor, *s*, with two lysineless strains have been prepared and tested. Several of these show growth responses, not characteristic of the

single mutants involved, which indicate a relationship in the metabolism of pyrimidines, lysine and arginine. Combination of the genes, lysineless *3*, pyrimidineless *3a* and *s* introduces a requirement for arginine. Lysineless strain *1* is able to use α amino adipic acid as effectively as it uses lysine. Combination of this gene with the suppressor gene *s* produces a strain which has the same requirement for lysine as lysineless *1*, but a much higher requirement for α amino adipic acid, unless arginine, citrulline or ornithine is also supplied. By combination of this lysineless gene with pyrimidineless *3a* a strain is produced which cannot use α amino adipic acid unless a small amount of lysine is added.

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[1] Houlahan, M. B., and Mitchell, H. K., PROC. NAT. ACAD. SCI., **33**, 223 (1947).

[2] Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **32**, 678 (1945).

[3] Doermann, A. H., Doctor of Philosophy, Dissertation, Stanford University, California (1946).

[4] Borsook, H., Deasy, C., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, **173**, 423 (1948).

[5] Mitchell, H. K., and Houlahan, M. B., *J. Biol. Chem.*, in press (1948).

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

[7] Doermann, A. H., *J. Biol. Chem.*, **160**, 95 (1945).

[8] Srb, A. M., and Horowitz, N. H., *Ibid.*, **154**, 129 (1944).

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*THE CYTOGENETIC EFFECT OF SONIC ENERGY APPLIED SIMULTANEOUSLY WITH X-RAYS**

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It is generally agreed that the number of chromosome breaks initially produced by a given dose of x-rays is independent of conditions at the time of radiation. But the subsequent yield of observable chromosomal aberrations from a given number of initial breaks depends upon the ratio of those breaks which rejoin (and are not detectable) to those which do not rejoin or which rejoin in detectable new associations. The majority of the breaks produced by x-radiation, under normal conditions, seem to rejoin in their original position and do not form observable aberrations.[3]

Any factors which affect this process of rejoining will influence the yield of chromosomal aberrations obtained from a given x-ray dose. Several treatments used in conjunction with x-rays have been shown to increase (or decrease) the yield as compared with that obtained from the same dose