$$r \text{ (p.i., amp.)} = 0.02,$$

is in agreement with that conclusion.

[1] Current estimates are 23 and 26 kpc, respectively.
[2] Shapley, McKibben and Mohr, these PROCEEDINGS, 26, 326 (1940).
[3] Gaposchkin, S., *Ibid.*, 24, 1 (1938).
[4] Gaposchkin, C. P., *Ast. Jour.*, 52, 218–226 (1947).

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*THE p-AMINOBENZOIC ACID REQUIREMENT OF THE "SULFONAMIDE-REQUIRING" MUTANT STRAIN OF NEUROSPORA**

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Emerson and Cushing[1] isolated a strain of *Neurospora* which instead of being inhibited in growth by sulfonamides requires the drug for growth at high temperatures. It seemed as if the roles of *p*-aminobenzoic acid (*PABA*) and sulfanilamide (*SA*) were reversed,[2] the mold being poisoned by *PABA* and this action being antagonized by *SA*. The evidence suggested that *SA* took the place of *PABA* as a metabolite.

In experiments to test the growth-promoting activity of folic acid compounds for certain *Neurospora* mutants, we found that the sulfonamide-requiring (*sfo*) strain can grow in the complete absence of *SA*. The following different strains[3] were used: wild type, *p*-aminobenzoicless (*pab*) of Tatum and Beadle,[4] sulfonamide-requiring (*sfo*) and the double mutant *p*-aminobenzoicless, sulfonamide-requiring (*pab, sfo*). The results are summarized in table 1.

Pteroyl-triglutamic acid has no growth-promoting action at all. Pteroylglutamic acid and *p*-aminobenzoylglutamic acid show growth activity for the *pab* mutant. Their activity is about 1 to 2% of the *PABA* activity. Pteroic acid seems to be more active, having about 5 to 10% of the activity of *PABA*. At higher concentrations these three substances antagonize *SA* inhibition in a competitive manner. Pteridine was also found to be without activity.

The response of the double mutant is unusual. It requires normally both *PABA* and *SA* for growth. We found that it grows in the absence of *SA* when pteroylglutamic acid or *p*-aminobenzoylglutamic acid is supplied, only slightly when pteroic acid is supplied. In the presence of 10^{-4} *M SA* the growth is poor with the first two substances, but good with the third one. In the presence of 10^{-7} *M PABA* there is no growth with

TABLE 1

GROWTH OF DIFFERENT STRAINS OF *Neurospora* ON FOLIC ACID AND RELATED COMPOUNDS; +++ GOOD GROWTH, ++ FAIR GROWTH, + POOR GROWTH, − NO GROWTH

		COMPOUNDS ADDED ($10^{-6}M$)					
STRAIN	MEDIUM	NONE	PETEROYL-TRIGLUTAMIC ACID	PTEROYL-GLUTAMIC ACID	PTEROIC ACID	*p*-AMINO-BENZOYL-GLUTAMIC ACID	*p*-AMINO-BENZOIC ACID
Wild type	Minimal	+++	+++	+++	+++	+++	+++
	10^{-2} *M SA*	−	−	−	−	−	++
pab	Minimal	−	−	++	+++	+++	+++
pab, sfo	Minimal	−	−	++	+	++	−
	10^{-4} *M SA*	−	−	+	+++	++	+++
	10^{-7} *M PABA*	−	−	−	−	−	−
sfo	Minimal	−	−	−	−	−	−
	10^{-4} *M SA*	+++	+++	+++	+++	+++	++

any of the substances tested. The simple sulfonamide-requiring mutant does not grow on any of the folic acid compounds.

The action of all three folic acid compounds can be explained by their free arylamine content. Crystalline folic acid (pteroylglutamic acid) contains about 0.5 to 1% *PABA*, *p*-aminobenzoylglutamic acid about 0.1% and pteroic acid about 6%.[5, 6] The *PABA* activity of these compounds is in some instances somewhat higher than can be accounted for by the free arylamine content; it seems likely that a certain amount of cleavage to *PABA* occurs during autoclaving or during growth of the mold. From their inability to substitute for *PABA*, it may be concluded either that folic acid and its derivatives are not products of *PABA* metabolism in *Neurospora*, or else that they have failed to penetrate into the cell in these experiments.

As for the double mutant, we assumed that it grows only at very low *PABA* concentrations, such as those provided as impurities in folic acid. This hypothesis was confirmed by growing the double mutant at low *PABA* concentrations. It can grow well in a very narrow range of *PABA* concentrations (Fig. 1). If we compare the growth rate of the double mutant with that of *pab*, we see that from the lowest *PABA* concentration up both curves ascend at exactly the same slope to about 2×10^{-8} to 5×10^{-8} *M*, where they reach the optimum growth velocity. The curve for the double mutant drops rapidly above 5×10^{-8} *M* to show a typically *SA*-requiring growth at *PABA* concentrations higher than 2×10^{-7} *M*. The growth between 5×10^{-8} and 2×10^{-7} *M* is typically adaptive: the velocity improves with time, reaching the normal rate at the end. At higher concentrations of *PABA* the curve is very irregular, owing to differences in the degree of adaptation to the absence of *SA*, and to "reversions" caused by mutation.[7]

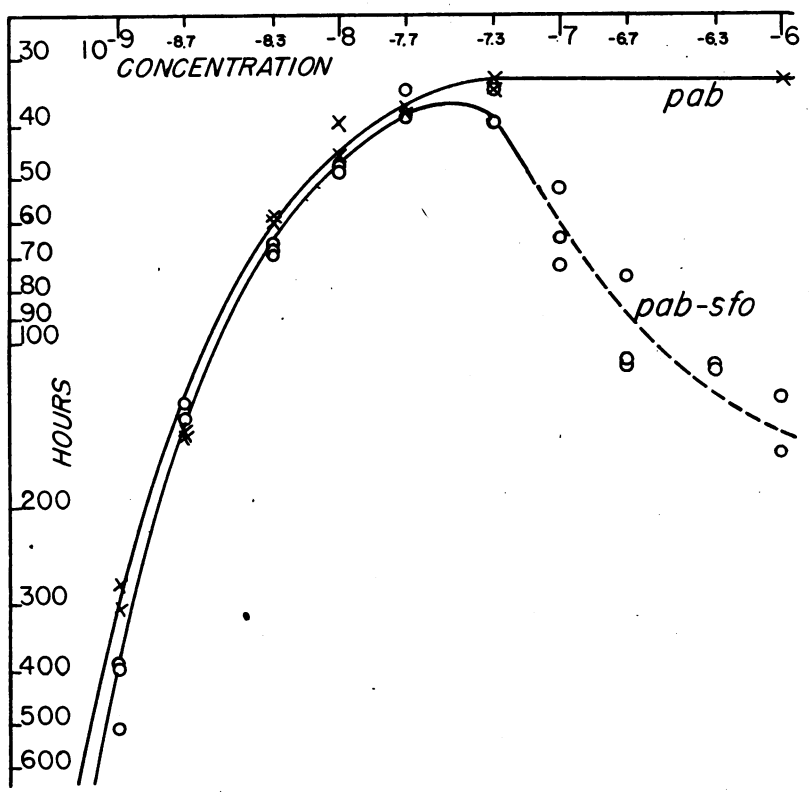

FIGURE 1

Growth of the *p*-aminobenzoicless mutant (*-x-x-*) and the double mutant *p*-aminobenzoicless, sulfonamide-requiring (*-o-o-*) on different concentrations of *p*-aminobenzoic acid (experiments Z — 90, Z — 101). Abscissa: concentration of *PABA* in moles; ordinata: time necessary for mycelium to reach 150 mm. in growth tubes.

Looking back to the action of the folic acid compounds, the observed results can be completely explained by the amounts of free *PABA* they contain. These amounts, as deduced from growth of the double mutant, are in agreement with the values obtained by assay with the *p*-aminobenzoicless mutant.

There is only one simple explanation of these facts: in the presence of the gene conditioning the *SA* requirement, *PABA* is poisonous in concentrations which are harmless to the wild type. As the inhibition by *PABA* (at concentrations less than 10^{-3} *M*) is completely relieved by *SA* in a competitive manner, we can draw a scheme comparable to the scheme of *PABA* action by Kohn and Harris.[8] They assumed that *PABA* is involved in the synthesis of substances x_1, x_2, $\ldots x_n$ which are necessary

for growth. *SA* competes with *PABA* and inhibits these syntheses. In our case the *sfo* gene would direct a reaction whereby *PABA* produces some inhibitory substance, *y*. This reaction must require a somewhat higher *PABA* concentration than is needed for normal growth and therefore is not apparent below a concentration of 10^{-7} *M PABA*. Further, the reaction must be very sensitive to *SA* inhibition and be stopped before *SA* inhibits growth by affecting vital reactions (leading to x_1, x_2, etc.). Search is under way for the particular reaction involved.

The sulfonamide-requiring mutant, in the absence of the *pab* gene, obviously produces more *PABA* than corresponds to a concentration of 10^{-7} *M*, so that it poisons itself and requires *SA* for growth as a detoxicant. Hence we have to drop the idea that *SA* is utilized as a metabolite.

Summary.—1. *Neurospora* cannot use pteroylglutamic acid, pteroic acid or *p*-aminobenzoylglutamic acid to replace *p*-aminobenzoic acid.

2. These folic acid compounds contain a certain amount of free *PABA*, which explains a positive growth response of the *p*-aminobenzoicless mutant and the double mutant *p*-aminobenzoicless, sulfonamide-requiring.

3. The double mutant can grow well on low concentrations of *PABA* (10^{-8} to 10^{-7} *M*), but is poisoned by greater concentrations and requires *SA* as a detoxicant.

4. The sulfonamide-requiring strain must produce more than the tolerated amount of *PABA* and thus inhibits itself.

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[3] The particular strains used are: wild type E5256A, *pab* 1633 reisolated as Z35-I-8, *sfo* E15172 reisolated as E16014A and *pab*, *sfo* 1633 and E15172 combined in strain E19335.

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DIRECTED FERTILIZATION IN MAIZE

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Ordinarily the two gametes in the maize pollen grain are genetically identical. However, a plant carrying an A-B interchange produces a pollen grain in which the gametes are not alike.[1] This paper deals with the rôle of the dissimilar gametes in fertilization.

An A-B interchange is one between the supernumerary B-type chromosome[2] and a member of the basic complement (A-type chromosome). The behavior of the interchange chromosomes during the development of the pollen grain may be illustrated by the case of TB-4a. In this interchange, the distal seven-eighths or so of the short arm of chromosome 4 was transferred to a segment of a B-type bearing the centromere of the latter. The resulting chromosome is designated the B^4 chromosome. The other interchange chromosome (4^B) contains the rest of chromosome 4 and most or all of the distal heterochromatic segment of the B-type.

The 4^B chromosome is orthodox in its behavior and is found regularly in each gamete of the pollen grain. The B^4 chromosome also follows the normal pattern until the division of the generative nucleus is reached. In this division the B^4 chromosome frequently undergoes non-disjunction so that one of the gametes receives two B^4 chromosomes and the other receives none. A second type of pollen grain is the result of normal disjunction; both gametes of the pollen grain are identical, each containing one B^4 chromosome.

Three classes of seed are expected when a normal seed parent is crossed with a pollen parent homozygous for TB-4a. Two of these are obtained from fertilization involving the first type of pollen grain. If the gamete that is deficient for the B^4 chromosome fertilizes the egg and its partner unites with the polar nuclei, a seed with a deficient embryo and a hyperploid endosperm will result (Class I). If the gametes exchange their respective rôles in fertilization, the seed will have an embryo hyperploid for the B^4 chromosome and a deficient endosperm (Class II). A third kind of seed is produced when the second pollen type is involved; in this case, the embryo is heterozygous for the interchange and the endosperm also carries a single B^4 chromosome (Class III).