(which contains *tr-1*) is automatically excluded.

Since a total of 6 out of 24 of the isolates examined do not appear to be carrying recessive lethals as judged by genetic analysis, it is felt that this offers a partial explanation for the discrepancy between the Horowitz and Atwood-Mukai methods of ascertaining the proportion of indispensable genes. The aberrant heterokaryon mutants are thought to be semilethal genetic changes which will act as fully lethal in the debilitating genetic background employed by the nature of the heterokaryon method. However, when crossed to wild type the progeny spores will possess a new genetic background, and at least some of the asci will carry more than four viable spores. Although the Atwood-Mukai heterokaryon employed different genetic markers, many of their isolates were probably semilethal genes of this sort.

It should also be pointed out that 6 out of 24 is a minimum estimate for the fraction of semilethal genes present. The possibility exists that others were not separated from their debilitating background in the asci dissected and were classed as fully lethal.

If the hypothesis of a large class of semilethal genes among those mutants isolated by the heterokaryon method is correct, then one would expect that in unselected material slow-growing mutants that do not respond to complete medium would greatly outnumber biochemical mutants. This is indeed the case, as Mitchell, Mitchell and Tissières (*5*) have shown. Another prediction is that the effects of the semilethal gene could be observed among the spores from the aberrant mutant crosses. Only three strains were examined in this fashion and in one (S13A) segregation for a growth-retarding factor was observed. However, in the other two strains neither growth tests nor visual observation unambiguously demonstrated a semilethal factor.

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Insect Fertility: Inhibition by Folic Acid Derivatives

Abstract. *Folic acid antagonists inhibited oviposition by screw-worm flies* [Cochliomyia hominivorax *(Coquerel); Diptera: Calliphoridae*]. *Fertility was unaltered when sufficient folic acid was administered simultaneously, or it was partly restored spontaneously about 2 weeks after treatment. Folic acid antagonists principally affected only maturing eggs at any age, rather than those already matured.*

Impairment of fertility of screw-worm flies [*Cochliomyia hominivorax* (Coquerel); Diptera: Calliphoridae] by chemicals is being intensively investigated as an aspect of the method of insect control wherein the males are sterilized (*1*). Among several hundred compounds screened, several 4-amino derivatives of folic acid (*2*) were highly effective, manifesting their activity by inhibition of oviposition. More precise studies of the action of these antimetabolites are summarized in this report.

Bisexual or unisexual groups of adults less than 24 hours old were fed various concentrations (0.0001 to 10 percent) in honey or sugar syrup daily for 5 or 7 days, or for 1 day, after emergence. The relevant results are shown in Table 1. In addition, single treatments of both sexes with 0.05 percent aminopterin or chloromethotrexate limited the production of viable eggs to only 5 percent. Oviposition was inhibited by less aminopterin or methotrexate when both sexes were treated simultaneously than when females only were treated. Yet, when only males were treated, eggs hatched normally, or nearly so, even at a concentration of 5 percent. Treatment of male *Drosophila melanogaster* Meigen (*4*) or *Musca domestica* Linnaeus (*5*) similarly did not affect oviposition by untreated females. The weight of evidence does not support a conclusion that fertility is affected when males are treated.

Methotrexate and chloromethotrexate were more effective than the other two compounds. Each of the first two compounds has a methyl group attached to the *p*-benzoylamino group of aminopterin, a substitution markedly enhancing activity. However, although one chlorine atom added to the benzoyl ring of methotrexate hardly reduced efficiency, two chlorine atoms (dichloromethotrexate) reduced the activity to less than that of aminopterin. Generally, folic acid antagonists powerfully inhibit folic acid enzymes by interfering with intracellular nucleic acid synthesis of rapidly proliferating cells, which in turn leads to disturbances of cell division and to chromosomal damage (*3*). Nucleic acids undergo rapid synthesis in nurse cells of young female screw-worm flies, but they are already formed in postmeiotic male germ cells, some of which have already left the testes as mature motile sperm when the adult emerges from the pupa. Therefore, effects of metabolic antagonism can only be exerted in females. Antagonism of folic acid similarly occurs and leads to inhibition of oviposition in other insects, for example, *Drosophila* (*4*), *Musca* (*5*), and *Bracon hebetor* Say (*6*).

Flies fed 0.05 percent methotrexate and 20 percent folic acid simultaneously for 24 hours after emergence were normally fecund and fertile. The number of viable eggs produced increased as the concentration of folic acid was increased from 1 to 20 percent (Fig. 1). This increase was rapid up to a concentration of 3 percent folic acid, but lessened as the concentration increased. The action of methotrexate was nullified when 400 times as much folic acid as antimetabolite was used. The reversible affinity of folic acid reductase is far greater (up to 1000 times) for these antimetabolites than for the normal substrates, folic and dihydrofolic acids

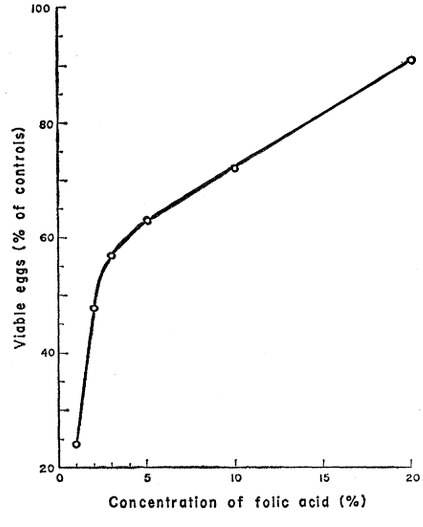

Fig. 1. Viable egg production by screw-worm flies treated orally with 0.05 percent methotrexate plus various concentrations of folic acid.

Table 1. Minimum concentrations (percent) of folic acid derivatives inhibiting reproductive capacity of screw-worm flies (two replicates).

Compound	Daily treatments (♂ and ♀)	One treatment (♂ and ♀)	One treatment (♀)
Inhibiting production of eggs			
Aminopterin	0.01	0.1	5.0
Methotrexate	0.001	0.05	0.5
Chloromethotrexate	0.001	0.5	0.1
Dichloromethotrexate	1.0	*	†
Inhibiting production of viable larvae			
Aminopterin	0.001	*	1.0
Methotrexate	*	*	0.1

* No inhibition. † Not tested.

Table 2. Oviposition by nonparous (two replicates) and parous (three replicates) screw-worm flies treated orally at different ages with 0.5 percent chloromethotrexate for 24 hours.

Age (days)	Females ovipositing (%)	
	Nonparous	Parous
1	0	*
3	75	*
7	50	10
10	*	0
14	45	3

* Not tested.

(*7*). This affinity accounts for the resistance of treated flies to restoration of normal fertility with large doses of folic acid. Mitlin *et al.* were unable to reverse the effect of aminopterin in house flies (*5*) because they used inadequate amounts of substrate.

Sterility induced by chloromethotrexate was not permanent. Virgin females, fed 0.5 percent chloromethotrexate for 24 hours after emergence, were given the opportunity to lay eggs without mating when 7 days old and every 3 or 4 days thereafter. A different group was allowed to oviposit for the first time on each of these occasions and again at 3- to 4-day intervals. (Maturation of normal ovarioles occurs synchronously; a gravid female may deposit 200 to 250 eggs in a shingle-like mass every 3 or 4 days.) No eggs were laid at the first two opportunities for oviposition, whether the first opportunity was at 7 or 23 days. From the time of the third opportunity for oviposition onward, production of eggs was considerably reduced (about 5 to 15 percent of controls). In a similar experiment, females treated after emergence and mated with normal males did not lay eggs when first permitted to oviposit 6 or 9 days later. Oviposition (less than 15 percent of controls) occurred on and after the 13th day; fewer than 10 percent of the eggs hatched. Moreover, some fertility was restored by the time second egg masses matured at any age. Perhaps insufficient unbound chemical remained to inactivate completely the second and third egg masses of mated and unmated flies, respectively. Spontaneous recovery from inhibitory effects of folic acid antagonists apparently also occurs in *Drosophila* (*4*), *Bracon* (*6*), and *Musca* (*8*).

Thus, mating evidently influenced egg deposition. Unmated females never oviposited at the first or second opportunity, but mated females always produced a first egg mass from the 13th day onward and usually a second mass irrespective of age. Eggs were not laid by 80 percent of unmated and 30 percent of mated flies (among 595 and 277 oviposition opportunities, respectively, of 197 unmated and 167 mated flies).

Table 2 shows the relation of oviposition by treated 7- and 14-day-old females to mating and parity. Only the youngest virgin (nonparous) females did not lay eggs 7 days after treatment and without mating; about half to three-quarters of older flies oviposited normally. Production of viable eggs by normally fertile, mated (parous) females at the three ages was less than 1 percent of controls 7 days after treatment. (Prior to treatment, 87 percent laid eggs, of which 84 percent hatched.) Not more than 20 percent as many parous as nonparous females laid eggs. Hence, chloromethotrexate retained more than 99 percent activity during the maturation of the egg mass of flies that had been treated at 7 or 14 days of age. Although comparison of nonparous and parous females is made between first and second egg masses, respectively, virgin females treated upon emergence in earlier tests were equally infecund at the first two opportunities of oviposition. Chloromethotrexate no longer completely inhibited oviposition from the 3rd day on, at least in nonparous females.

Apparently, activity of folic acid and its antimetabolites is exerted during metabolic processes of egg maturation. Moreover, ovarian growth and egg production are inhibited by physical or chemical agents much more readily during the endomitotic phase of nurse cells than at 24 hours or later when this phase is completed (*9*). The present data reflect these relative susceptibilities of the ovary to inhibitory agents. The work of Grosch (*6*) similarly shows the relation of methotrexate inhibition of oviposition in *Bracon* to atrophy of nurse cells and degeneration of oocytes.

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Mutant Mice (Quaking and Jimpy) with Deficient Myelination in the Central Nervous System

Abstract. *Two mutant mice with deficient myelination are described. Quaking is a new autosomal recessive mutant mouse with marked tremor of the hindquarters. The mice eat, swim, breed, and nurse well even though the entire central nervous system is very deficient in myelin by histological and chemical criteria. Myelin formation is impaired; no destruction is seen. Peripheral nerves are myelinated. Jimpy, a known sex-linked mutation, has similar but more severe symptoms and similar pathology, with the additional feature of sudanophilic (nonpolar) lipid distributed in some white-matter tracts. Both mutants offer new opportunities for study of the formation and functions of myelin.*

Mammalian mutants are of great value for the study of development, structure, function, and disease of the nervous system. Yet few of the more than 30 different neurological mutants now available have been well characterized, and more mutants are rec-