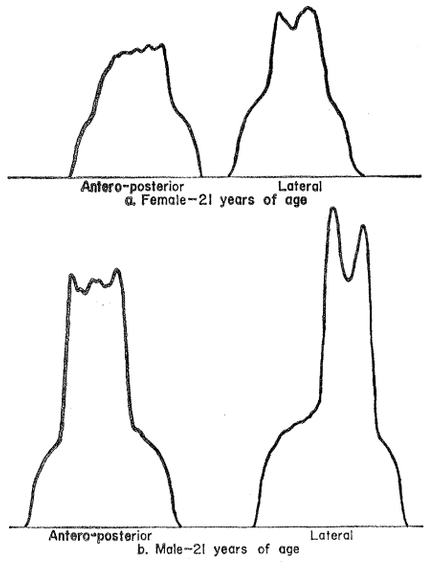

Fig. 2. Finger traces made by the bone densitometer. (*a*) Normal female, 5 feet 3½ inches (1.613 m) tall, 116 lb (53 kg) body weight, and 21 years of age, whose dietary history indicated a low calcium intake. Her present mean calcium intake is 0.55 g/day with a mean protein intake of 74.2 g/day. Her bone density index is 0.632. (*b*) Normal male, 5 feet 9 inches (1.753 m) tall, 184 lb (83 kg) body weight, and 21 years of age, whose dietary history indicated a high calcium intake. His present mean calcium intake is 2.96 g/day with a mean protein intake of 111.5 g/day. His bone density index is 1.429.

mined by making repeated measurements on a phantom finger. The phantom finger was made of a section of porcine rib encased in plastic (*4*). The plastic had a density approximating flesh density. With the phantom finger, results of the bone densitometer are reproducible within 3 percent. Repeated measurements on human subjects are reproducible within 6 percent, as compared with 12 to 14 percent when film is used.

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2. The instrument was developed under the direction of Dr. D. E. Williams (now retired) and was built by Spinlab, Inc., Knoxville, Tenn.
3. D. E. Williams and R. L. Mason, *Science* **138**, 39 (1962).
4. Trade name of the plastic is Castolite.
5. We thank H. C. Wang for his help in checking the instrument. This work was supported in part by grant AM-03345 from the PHS.

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Morphogenetic Effects of 6-Azauracil and 6-Azauridine

Abstract. *When 6-azauracil and 6-azauridine are fed to larvae of the* tu^w rc *strain of* Drosophila melanogaster *which has the normal wing shape, adults hatch with shortened, obliquely truncated wings. This wing shape resembles that of the mutant* dumpy. *On the other hand, administration of these drugs to the mutant* dumpy *strain increases wing length, and flies with normal wings are obtained.*

Studies on the biological effects of azapyrimidines have been directed toward their use as bacteriostatic and cancerostatic agents. The synthesis of pyrimidine bases is inhibited by both 6-azauracil and 6-azauridine, their antimetabolic effectiveness being dependent on their conversion to 6-azauridine-5′-phosphate which affects the activity of orotidylic acid decarboxylase (*1*). Since the penetrance of melanotic tumors in tumorous strains of *Drosophila* is influenced by the amount of nucleotides and nucleosides included in the larval diet (*2*), a series of experiments was undertaken to study the effects, if any, of 6-azauracil and 6-azauridine on the development of melanotic masses in *Drosophila* larvae. During this survey phenotypic effects on wing development in *Drosophila* were noted, and these observations are summarized here.

Larvae of the *tumor^w* (*tu^w*) strain of *D. melanogaster* develop melanotic masses in the caudal fat bodies shortly before pupation (*3*). The melanotic masses survive pupal metamorphosis and are retained during adult life as inert black bodies in the abdomen. Otherwise, adults of the *tu^w* strain are morphologically comparable to the wild-type *Ore-R* strain. For our experiments, a *tu^w* stock which also contained the mutant allele *red cell* (*rc*) was used, since viability and tumor penetrance in this particular stock excel those in our stock of *tu^w*. Eggs and larvae of the *tu^w rc* stock were collected at 24°C; larvae were maintained on the usual cream-of-wheat medium for *Drosophila* until they were 67 to 68 hours of age. The larvae were then transferred to paper pulp which was moistened with the various solutions used in each experiment. Since solutions of azauracil and azauridine were prepared in distilled water, the larvae used as controls were, in each experiment, transferred to paper pulp moistened with distilled water. Larvae of *D. melanogaster* will continue development if removed from food after 65 hours of age, and normal adults, although slightly smaller in size, will emerge. A series of concentrations of 6-azauracil and 6-azauridine were tested in preliminary experiments. At a concentration of 0.5 mg/ml, 6-azauracil gives a mortality of 0.25 ± 0.034 (number of larvae, N, = 179), and 0.29 ± 0.026 ($N = 278$) of the treated *tu^w rc* larvae survive a concentration of 1 mg of 6-azauracil per milliliter. Azauridine equimolar with the former concentration of azauracil produces a mortality of 0.12 ± 0.017 ($N = 347$).

The wing shape of *tu^w rc* adults is the same as that of the wild-type *Ore-R* strain, and Fig. 1*A* demonstrates the appearance of the wing of a *tu^w rc* control specimen transferred to H_2O when the adults are 67 hours of age. After the feeding of azauracil or azauridine, larvae were obtained with an oblique shortening of the wing (Fig. 1*B*). This wing shape closely approximates that of the known mutant, *dumpy* (*dp*) (Fig. 1*C*). Various degrees of this effect are obtained, some wings showing a shortening as extreme as that of the *dp* mutant and other wings displaying a truncation of the 1st and 2nd posterior cells with the wing appearing pointed. Both wings of a fly are affected to a similar degree, and only an occasional disparity between the wings of an affected individual is noted. After treatment with azauridine (1 mg/ml), 23 percent of the individuals hatching ($N = 238$) showed effects on the wing; 44 percent of the hatching adults ($N = 57$) showed wing abnormalities after administration of 0.5 mg of 6-azauracil per milliliter; and 83 percent of the surviving individuals ($N = 24$) were affected by feeding 1 mg of 6-azauracil per milliliter.

The term phenocopy designates an artificial phenotypic effect which resembles the phenotypic expression of a known mutant. The morphological abnormality produced by azauracil and azauridine resembles that of the mutant phenotype of *dp*. However, the underlying mechanism in phenocopy production may not be the same as that involved in the action of the mutant gene *dp*. If the mechanism of phenocopy production parallels the action of the mutant gene, treatment of the mutant strain with these chemicals would

be expected either to increase the *dp* wing effect or to have no effect on the expression of the mutant gene. Azauracil and azauridine were therefore fed to the mutant *dp* strain to determine whether mortality of this strain would differ from that of the tu^{w} *rc* strain or whether wing morphology would be altered. When *dp* larvae are transferred to water at 67 hours of age they pupate, and expression of the *dp* phenotype is 100 percent (Fig. 1*C*). When *dp* larvae are given azauracil or azauridine, flies with normal wings are obtained. Again, there is variability of response, some wings being indistinguishable from the wild-type normal wing while other flies have wings in which the *dp* truncation is only slightly reduced (50 affected out of 109 survivors). Both wings of an affected fly generally show an equal degree of wing normality, but some individuals possess one wing which has attained normal dimensions, whereas the other has remained slightly truncated (example in Fig. 1*D*).

Development of the wing in the *dp* mutant of *Drosophila* has been studied by Waddington (*4*) who concluded that the mutant wing shape is the result of an increased contraction of the wing during pupal development. Goldschmidt (*5*) had earlier suggested that the decreased longitudinal size of the *dp* wing might result from degeneration of the cells in the distal region of the wing, but this explanation was not accepted by Waddington who failed to locate degenerating cells in *dp* wings. Morphogenesis of the wing in the *dp* mutant and the "dumpy" phenocopy must be compared to determine how closely the cellular pattern of phenocopy production parallels the expression of the genotypic activity of the mutant allele at the *dp* locus. Azauracil or azauridine have not induced morphogenetic effects in the *Ore-R* (wild-type) strain nor have heterozygous larvae (*Ore-R*/tu^{w} *rc*, *Ore-R*/*dp*, *dp*/tu^{w} *rc*) given any response. Thus the differences in response to treatment with azapyrimidines are genetically determined, and it is unlikely that the "dumpy" phenocopy in the tu^{w} *rc* strain is due to the presence of a subthreshold allele at the *dp* locus in this stock.

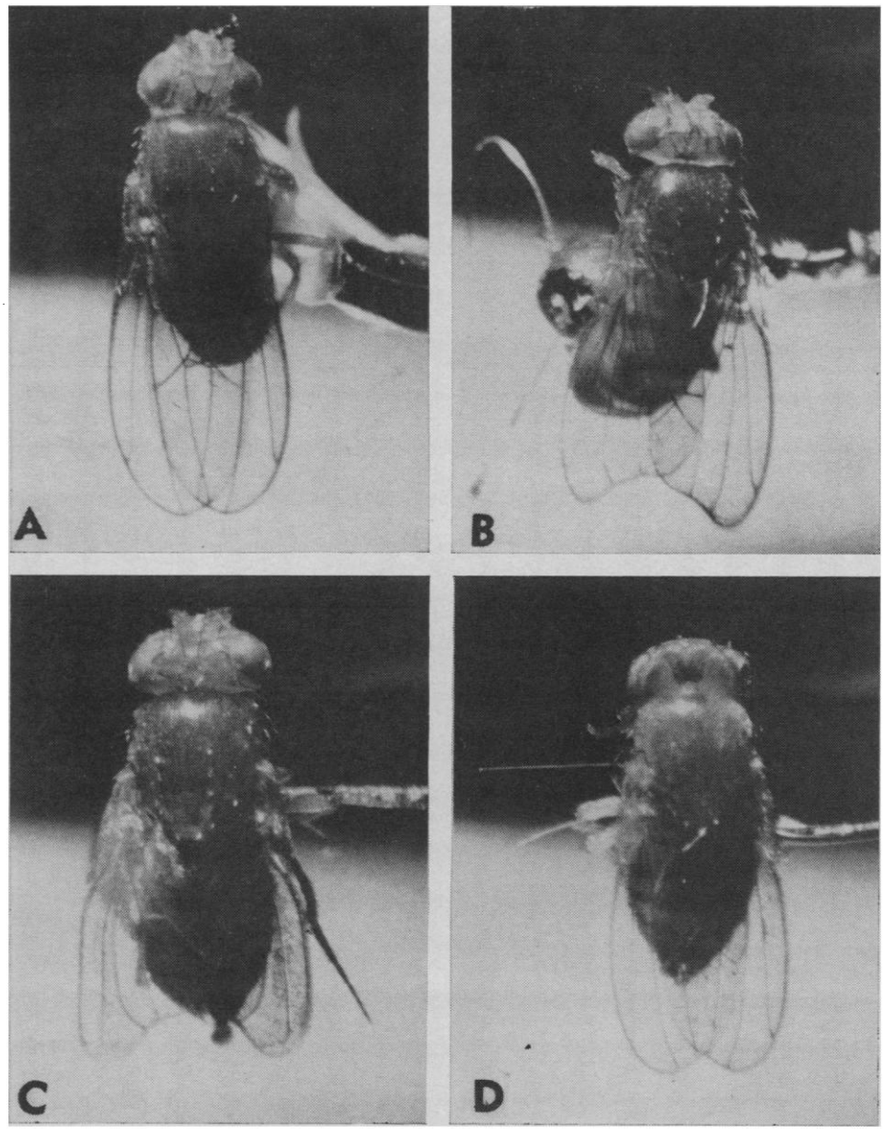

Fig. 1. (*A* and *B*) Adult males of the tu^{w} *rc* strain of *D. melanogaster*: (*A*) control specimen transferred to water at larval age of 67 hours; (*B*) transferred to azauridine at larval age of 67 hours. The distal margin of the wing differs from the control specimen and resembles the wing shape of the *dp* mutant photographed in Fig. 1*C*. (*C* and *D*) Female specimens of the *dp* mutant strain: (*C*) control specimen transferred to water at larval age 67 hours; (*D*) transferred to azauridine at larval age 67 hours. Treatment with azauridine affects the mutant phenotype, and the wing shape resembles that of the fly in Fig. 1*A*.

Our observations reveal an anomalous effect, for, in one case, azauracil and azauridine are initiating a lengthening of the wing, and in the other mutant, a retardation of the lengthening process appears. These effects seem to have an intimate relationship, because the site of morphogenetic response is the distal region of the wing in both instances. Such an antagonistic effect might indicate that wing morphogenesis is controlled by a series of genetically determined competitive steps in which the amount of RNA synthesis is a limiting factor. A shift in the rates of interrelated steps might set morphogenesis into alternative pathways. Indeed, the concept of mutant genes controlling the rates of morphogenetic processes as formulated by Goldschmidt (*5*) might aptly be used in analyzing the effects of azauracil and azauridine on wing morphogenesis.

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