concentrations (10^{-9} to $10^{-6}M$) of warfarin have no effect on slices from normal animals, because slices are not viable for a period sufficient to deplete them of the vitamin K already at the site of action. The results reported here, therefore, indicate the need for a reinterpretation of the significance of previous studies of the effects of warfarin and other indirect anticoagulants on isolated tissue preparations.

Finally, it has not escaped our notice that in slices from vitamin K–deficient animals, warfarin appears to inhibit the release of factor VII without inhibiting protein synthesis. However, this does not necessarily indicate that the appearance of factor VII requires no *de-novo* protein synthesis. Since the synthesis of factor VII and other vitamin K–dependent clotting factors probably is only a very small fraction of the total protein synthesis carried out by the liver, specific inhibition of factor VII synthesis might not have been detected by the method used to measure total protein synthesis. Other workers (*10–13*), studying the effects of specific inhibitors of protein synthesis in intact animals and in in vitro systems on the response to vitamin K, have failed to show conclusively whether the response depends on *de novo* protein synthesis or on the transformation and release of precursors of clotting factors.

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Crystal and Molecular Structure of a Thymine-Thymine Adduct

Abstract. *Thymine-thymine adduct is a product isolated from thymine irradiated with ultraviolet light in frozen aqueous solution. This compound is presumably formed through the rearrangement of an initial photoproduct. Single crystal x-ray diffraction analysis has confirmed the molecular formula of the adduct, 5-hydroxy-6-4′-(5′-methylpyrimid-2′-one)-dihydrothymine, except for the possibility of a hydrogen atom on the 3′ nitrogren rather than the 1′ nitrogen, and has established the stereoconfiguration of the molecule.*

Ultraviolet irradiation of frozen aqueous solutions of thymine and of DNA has yielded cyclobutane-type dimers of thymine (*1*). Recently, a different kind of thymine-thymine product has been isolated from the ultraviolet irradiation of frozen aqueous solutions of thymine (*2*). From spectroscopic evidence the product was deduced to be 5-hydroxy-6-4′-(5′-methylpyrimid-2′-one)-dihydrothymine (*2*). For proof of the structure, a crystal of the adduct was subjected to an x-ray diffraction analysis.

X-ray diffraction data for a single crystal of the thymine-thymine adduct were collected photographically about two axes by the multiple film, equi-inclination Weissenberg technique for a total of 1844 independent reflections. The material crystallizes in the centrosymmetric triclinic space group of $P\bar{1}$ with two molecules per unit cell. The cell parameters are as follows: a, 9.44± .02 Å; b, 8.29 ± .02 Å; c, 7.57 ± .02 Å; α, 99.0°; β, 91.5°; and γ, 89.8°.

The crystal structure was solved directly by the symbolic addition procedure (*3*), and all the atoms were located on the first E-map. The coordinates and thermal parameters were refined by least-squares methods and the hydrogen atoms were found in a difference map. The agreement between observed and calculated structure factors is 10.2 percent. The molecular structure (Fig. 1) shows that the methyl group on carbon-5 of ring I and ring II on carbon-6 of ring I are both axial to ring I. The structure analysis indicates that an H atom is located on the 3′ nitrogen rather than the 1′ nitrogen.

Since the material crystallizes in a centrosymmetric system, the two molecules in the unit cell are a racemic pair. Ring II is planar to within ± .02 Å, while ring I is in the half-chair conformation with carbon-5 0.4 Å below and carbon-6 0.2 Å above the plane of the other four atoms. The angle of inclination between the two rings is approximately 96° (Fig. 2). Bond lengths in the dihydrothymine moiety are quite similar to those found for the molecules of dihydrothymine (*4*) and dihydrothymidine (*5*). A water of hydration crystallizes with the T-T adduct and is included in an extensive system of hydrogen bonding in the crystal.

The complete elucidation of the stereoconfiguration of T-T adduct proves its molecular structure and may

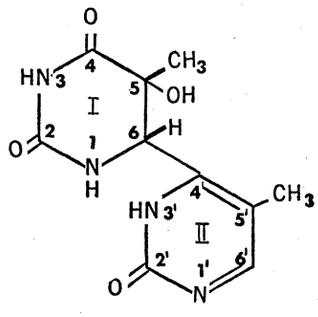

Fig. 1

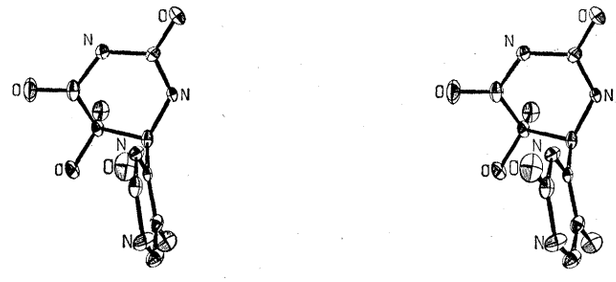

Fig. 2. Stereodiagrams of the configuration of thymine-thymine adduct as determined by x-ray analysis. The picture should be seen with a three-dimensional viewer for printed stereophotographs (*7*).

enable us to have a better understanding of the chemistry of the T-T adduct as it relates to photobiology (*6*).

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Mosquitoes Feeding on Insect Larvae

Abstract. *Caged* Aedes aegypti *and* Culex tarsalis *are attracted to insect larvae, engorge on their body fluids, and produce viable eggs. Attractiveness of the larvae is related to their size, shape, and color but not to their movement. The possibility that wild mosquitoes substitute insect hemolymph for vertebrate blood is discussed.*

Serological analyses of their gut content show that mosquitoes attack most groups of vertebrates, some species feeding selectively on particular hosts [for example, *Culex territans* Walk. on amphibians and reptiles (*1*)], and others on many taxa [for example, *Aedes aegypti* (L.) (*2*)]. However, the few records of mosquitoes feeding on insects have been discounted by Downes (*3*), and it is generally assumed that the nourishment needed for egg development is not supplied by the body fluids of invertebrates. Our results, by contrast, show that in the laboratory some mosquitoes feed on insect larvae and produce viable eggs as a result; they are attracted to the larvae and evidently recognize them as hosts.

Laboratory-propagated *Aedes aegypti* and *Culex tarsalis* were kept in cages, 22 by 22 by 37 cm (approximately 150 females and 50 males to a cage), at 26°C, 65 percent relative humidity, and a 14-hour photoperiod (14 hours light and 10 hours dark). Honey solution (10 percent) was available at all times. Host acceptance by *Aedes aegypti* (Table 1) was determined by placing invertebrates—either living or coddled (that is, previously immobilized in warm water)—in the cages and by watching for at least 2 hours to see if feeding occurred. The living invertebrates were placed in the cage with the mosquitoes, and the coddled insects were tied to the cage walls. The comparative attractiveness of various insect features was investigated with the use of fifth-instar *Galleria mellonella* (L.), and third-instar *Celerio euphorbiae* (L.) of similar size; living, coddled, and stuffed larvae (that is, those whose body contents had been replaced with latex) were offered in paired cages and observed for 30 minutes to determine the landing rate on each (Table 2). Mosquitoes that fed were transferred for oviposition to clear plastic tumblers inverted over wet filter paper.

Figure 1 shows *Aedes aegypti* feeding on a coddled *Celerio euphorbiae* larva. The *Aedes aegypti* also fed on larvae of Coleoptera, Hymenoptera, and other Lepidoptera but not on Neuroptera, spiders, or earthworms (Table 1). A similar test showed also that *Culex tarsalis* fed on coddled *Celerio euphorbiae*, with a landing rate of 30 per hour. However, in contrast to *Aedes aegypti*, which fed on insects within 4 days of its emergence, *Culex tarsalis* did not do so until at least 2 weeks old although they would accept red-blood meals during this period. Also, unlike that of *Aedes aegypti*, the stock of *Culex tarsalis* was partially autogenous; therefore the evidence that *Culex tarsalis* had used hemolymph for egg production was not conclusive. The *Aedes aegypti* fed less successfully on some species of larvae than on others. For example, the violent reactions of *Celerio euphorbiae* when probed usually dislodged the mosquitoes before they could feed. In contrast, *Euxoa messoria* (Har.) merely curled up and remained motionless, so that the mosquitoes could probe and feed without disturbance, although they had difficulty in penetrating the cuticle of this species. Immobilization of the larvae by coddling usually enabled more mosquitoes to feed and larger meals to be taken. However, persistent individual mos-

Table 1. Acceptance of coddled invertebrates by *Aedes aegypti* in the laboratory.

Test host	Landing rate*	Response	
		Feeding	Eggs laid†
Arthropoda			
Lepidoptera			
Celerio euphorbiae (L.)	74	X	X
Manduca quinquemaculata (Haw.)	76	X	0
Calophasia lunula (Hufn.)		X	X
Euxoa messoria (Har.)	57	X	X
Danaus plexippus (L.)	55	X	0
Galleria mellonella (L.)	10	X	X
Coleoptera			
Leptinotarsus decemlineata (Say)	42	X	0
Hymenoptera			
Neodiprion lecontei (Fitch.)		X	X
Neuroptera			
Corydalis cornutus (L.)	0	0	0
Araneida			
Argiope aurantia Lucas *Araneus trifolium* (Hentz)	6	0	0
Annelida			
Oligochaeta			
Lumbricus sp.		0	0

* Number of mosquitoes (both sexes) landing during 1 hour on five host specimens except for the spiders (Araneida) in which two specimens (one of each species) were tested together. † Laid eggs were viable.