properties of murine C-type viruses. We do not yet know if the virus induced from normal BALB/3T3 is different in any of its in vivo or in vitro properties from those viruses induced from "transformed" cells.

The mouse cell lines described here may be comparable to lysogenized bacterial cells. The nature of the control of expression of virus genetic and oncogenic information remains to be determined. The ability of agents such as BrdU to induce the formation of C-type viruses in well-characterized clonal lines should permit study of the regulation of endogenous tumor virus information. Such studies have obvious implications for chemical and viral carcinogenesis.

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Chemosterilant Action of Anthramycin: A Proposed Mechanism

Abstract. *The activity of anthramycin and structurally related analogs as chemosterilants of the housefly,* Musca domestica *L., correlates closely with the action of these compounds as inhibitors of* Escherichia coli *RNA polymerase. Since inhibition of RNA polymerase by anthramycin reflects binding of this antibiotic to the DNA primer required for enzyme activity, we propose that the interaction of anthramycin with DNA may also account for its action as a chemosterilant.*

The use of sterilized insects (*1*) has received considerable attention as an effective and safe means of insect control (*2*). The release of sterile males into the general population has already proved effective in controlling the screwworm fly in the southwestern region of the United States and in preventing the entrance of the Mexican fruit fly into southern California (*3*). Sterilization for these purposes is achieved by exposure of insects to gamma irradiation or by treatment with specific chemical agents. The latter approach, known as chemosterilization (*4*), has been proposed as a general means by which vertebrate, as well as invertebrate, populations can be controlled.

Insect chemosterilants include alkylating agents, antimetabolites, and various miscellaneous compounds (*5*). Although alkylating agents may induce chromosomal aberrations (*6*), the biochemical mechanisms by which chemosterilants affect fertility in insects has not been established.

Barnes *et al.* (*7*) recently described

Table 1. Effect of anthramycin and its derivatives on fertility and mortality of adult houseflies. Groups of ten newly emerged males or eight females were injected with various doses of the test compounds dissolved in a mixture of acetone and dimethylsulfoxide (1 : 1). The treated males or females were then caged with eight untreated virgin females or ten males of the same age. In all experiments, one or more batches of eggs were collected and their hatchability was scored as previously described (*13*).

Compound injected*	Dose (micrograms per fly)	Male injected			Female injected		
		Mortality† (%)	Eggs collected	Hatching (%)	Mortality† (%)	Eggs collected	Hatching (%)
None		0	1230	98			
I	0.25	0	740	5	0	103	79
	.50	10	362	0	0	0‡	
	1.00	100			100		
II	0.25	10	858	9	13	0‡	
	.50	30	300	1	13	0§	
	1.00	80	295	0	62	0§	
III	0.25	10	725	23	0	0‡	
	.50	20	137	0	38	0§	
	1.00	90	85	0	88	0§	
IV	0.25	0	527	53	0	480	79
	.50	0	638	43	0	377	74
	1.00	0	512	4	0	0‡	
V	0.25	0	332	92	0	541	82
	.50	0	509	93	0	371	88
	1.00	0	658	69	0	323	77
VI	0.25	0	567	94	0	456	96
	.50	0	744	91	0	659	93
	1.00	0	740	95	0	396	90
VII	1.00	0	483	96	0	287	90
VIII	0.25	0	452	94	13	515	85
	.50	0	662	86	0	486	88
	1.00	0	513	86	0	491	98
IX	0.25	0	536	85	0	642	88
	.50	20	672	86	0	665	84
	1.00	0	343	92	0	312	83

* Roman numerals refer to the structural formulas in Fig. 1. † Measurement at 48 hours. ‡ The female survivors were dissected 1 week after injection and their ovaries were found atrophied. § All injected females died before the date for egg collection (6 days after injection).

the sterilizing activity of the antitumor antibiotic, anthramycin (*8*), after ingestion of this compound by the female *Drosophila melanogaster* Meigen. We have subsequently shown that anthramycin is also a highly effective male chemosterilant when administered by intrathoracic injection to the male housefly, *Musca domestica* L. (*9*).

Studies on the mode of action of anthramycin suggest that it inhibits growth of certain bacterial and animal cells by binding to DNA, thereby interfering with the synthesis of nucleic acids (*10, 11*). This inhibitory action could account for its chemotherapeutic properties as an antitumor and antimicrobial agent (*12*). The experiments described in this report were designed to test the hypothesis that chemosterilant activity of anthramycin also results from the ability of this antibiotic to complex to DNA. We have, therefore, compared the structure-activity relations of anthramycin and its congeners as inhibitors of *Escherichia coli* RNA polymerase, which reflect the interaction with DNA, with their chemosterilant activity in *M. domestica.*

The structures of anthramycin and structurally related analogs are shown in Fig. 1, and their chemosterilant activity is summarized in Table 1. Anthramycin (I), epianthramycin (II), and anhydroanthramycin (III) were extremely effective in sterilizing male houseflies. All three compounds reduced hatching to minimal values when injected at a dose of 0.25 μg per fly. The estimated SD_{50} (the amount of drug that reduces hatching by 50 percent) for anthramycin in the male housefly is 0.03 μg per fly, compared to 0.1 μg per fly for the established chemosterilant TEPA [tris(1-aziridinyl)-phosphine oxide] (*13*). Intrathoracic administration of compounds I, II, or III at a dose of 1 μg per fly proved to be lethal, but injection of 0.25 μg per fly produced no more than 10 percent mortality. The nitrile (IV) also showed activity as a chemosterilant, reducing the rate of hatching to 53 and 4 percent, when administered to the male at a dose of 0.25 μg and 1.0 μg per fly, respectively. This analog showed no lethal effects at these concentrations.

Table 2. Effect of anthramycin and its derivatives on the activity of *E. coli* RNA polymerase. [*Escherichia coli* RNA polymerase previously described as ammonium sulfate fraction III (*15*), was a gift from Dr. Maitra.] The activity of this enzyme was determined, as previously described (*10*), in a standard reaction mixture containing tris-HCl (*p*H 7.5), 20 μmole; $MnCl_2$, 1 μmole; 2-mercaptoethanol, 1 μmole; 55 nmole each of adenosine triphosphate, uridine triphosphate, and cytidine triphosphate; [^{3}H]guanosine triphosphate (25 μc/μmole), 40 nmole; native calf thymus DNA, 10 μg; and 0.5 unit of enzyme in a final volume of 0.25 ml. Inhibitors were present at a concentration of $10^{-4}M$.

Compound*	Inhibition (%)
Anthramycin (I)	>75
II	>75
III	>75
IV	>50
V	<10
VI	<10
VII	<10
VIII	<10
IX	<10

* Roman numerals refer to the structural formulas in Fig. 1.

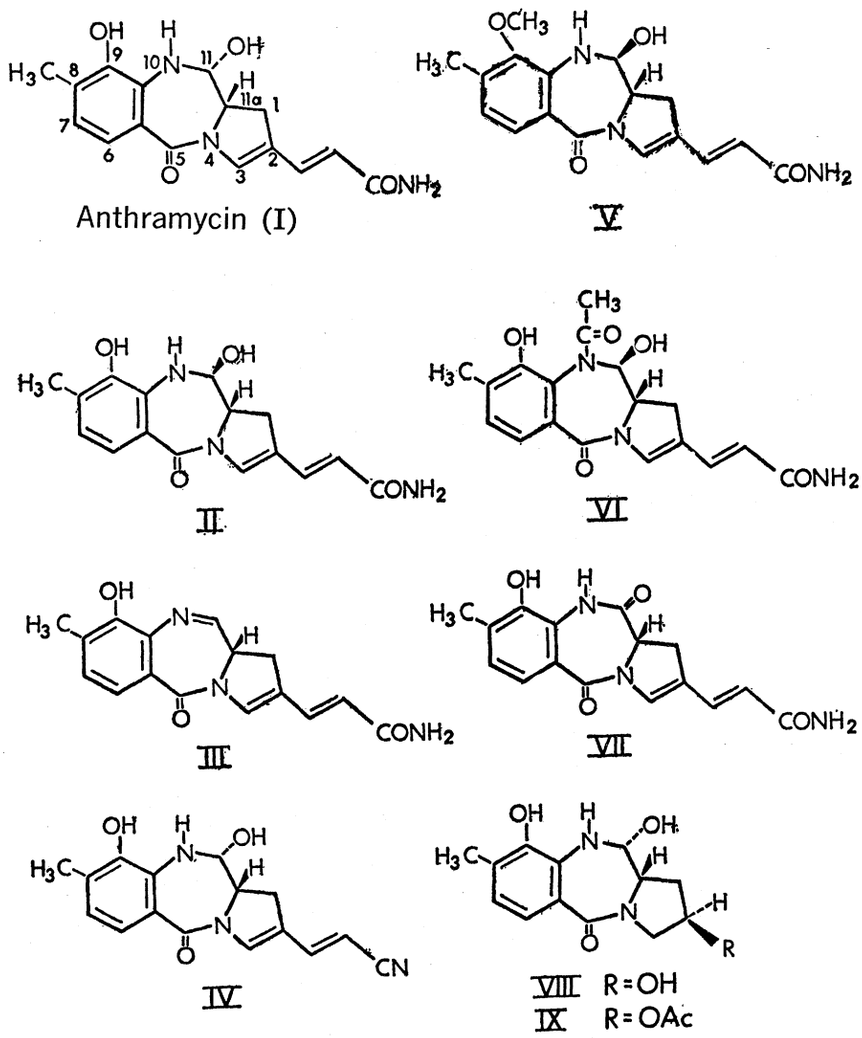

Fig. 1. Structural formula of anthramycin and related derivatives.

In the female housefly, lethal effects were observed within 48 hours when compounds I, II, and III were injected at a dose of 1.0 μg per fly. Administration of 0.5 μg of these compounds per fly produced ovarian atrophy or death of the injected females before egg collection on the seventh day. Anthramycin (I) had no lethal effects on female houseflies when a dose of 0.25 μg per fly was injected, and, in the limited number of eggs collected, the rate of hatching was reduced by only 21 percent. The nitrile (IV) induced ovarian atrophy at the highest dose tested (1 μg per fly), but lesser amounts had no significant effect on hatching. None of the other analogs tested (V to IX) had significant chemosterilant effects on the female.

Anthramycin and its analogs were also tested for their ability to inhibit the activity of RNA polymerase prepared from *E. coli* (Table 2). Inhibition of this enzyme activity provides a quantitative method for measuring the effects of these compounds on RNA synthesis in vitro. Greater than 75 per-

cent inhibition was produced by $10^{-4}M$ concentrations of either anthramycin (I), epianthramycin (II), or anhydroanthramycin (III). The nitrile (IV) retains significant inhibitory activity, while the other analogs tested (V to IX) are inactive.

The results of these structure-activity studies indicate that the ability of anthramycin and its derivatives to act as chemosterilants in houseflies correlates closely with the inhibitory effects of these compounds on the RNA polymerase of *E. coli*. In both assays, only anthramycin (I) and the closely related derivatives II, III, and IV are active. Analogs in which the phenolic function is methylated (V), the aniline nitrogen is acetylated (VI), the carbinol-amine function is replaced by an amide group (VII), or in which the conjugated side chain is absent (VIII and IX) are devoid of the sterilizing effects of anthramycin. The activity observed with compounds II and III was not unexpected, since the three forms are in rapid equilibrium with anthramycin (I) in aqueous solution (*14*). The other active analog (IV) differs from anthramycin in that the primary amide group is replaced by a nitrile function.

If the cytotoxic activity of anthramycin against animal cells and bacteria is correctly attributed to the interaction of this antibiotic with DNA [see (*10*, *11*)], the chemosterilant effect on adult flies may also relate to binding of DNA by this alkaloid. This interpretation is consistent with an effect on gene expression, which is usually responsible for chemosterilant activity. Selective effects on the male are of special interest for purposes of insect pest eradication, but further studies are needed to precisely define a molecular basis for the chemosterilant action of anthramycin.

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Synchrony and Flash Entrainment in a New Guinea Firefly

Abstract. *Fireflies can duplicate both faster and slower rhythms of artificial light. Since the interval between the pacer signal and the firefly's flash of the next cycle approximates the firefly's normal free-run period, it is suggested that the pacer signal resets the flash-timing oscillator in the brain, thus providing a mechanism for synchronization.*

Males of many species of fireflies flash spontaneously in a regular rhythm. Each flash is preceded by a volley of action potentials detectable in the main nerve trunk (*1*). Experiments with ablation and local excitation indicate that the neural timing mechanism (timer) which controls periodic flashing is in the brain. Flashing can be both enhanced and inhibited by appropriate photic or electrical stimulation of the eye (*1–3*), but little is known about how the rhythm is generated. As have others (*4*), we shall postulate that the timer normally behaves as a spontaneous "relaxation oscillator" (*5*) in which excitability increases progressively to a threshold or triggering level at which the system discharges, returns to baseline excitability, and begins its next cycle.

In parts of the tropical Orient there are species of fireflies in which the males habitually congregate in large swarms and flash in unison. Many hypotheses as to how the individual timers are brought into step have been advanced (*6*), but the synchronization is only partly understood. In the Thai species *Pteroptyx malaccae* Buck and Buck (*7*) found the interval between earliest and latest individual flashes in a communal flash to be only about 30 msec, whereas the minimum delay for flash generation, even by neural stimulation near the light organ, was 55 to 80 msec. During a mass flash, therefore, the fireflies cannot be responding to each other directly. Rather, it was suggested, each firefly lengthens or shortens his next interflash period according to whether he had flashed earlier or later than the average during the previous concerted emission. It was thought, in other words, that each firefly must be capable of distinguishing flash sequence and of controlling the period of his endogenous timer (*8*).

The recent *Alpha Helix* Expedition to New Guinea (*9*) gave us opportunity to study firefly synchrony with high-speed multichannel recording. We describe here only the most basic form of entrainment, that of single males responding to rhythmic flashes of artificial (pacer) light, and in only one species, *Pteroptyx cribellata* (*10*), which has a normal or free-run period of close to 1000 msec at 25°C. The firefly was