($r = -.09$) nor for the caffeine group ($r = -.09$), regardless of consumption experience (high intake: $r = .14$; low intake: $r = -.07$). Trait anxiety did not show significant relationships with consumption levels in either group (range of r's $= -.37$ to $.20$).

These findings demonstrate that caffeine consumption has consequences for anxiety, muscle tension, and reaction time in nonclinical subjects. The relations among these variables are not simple. Recent abstinence from caffeine may cause increased anxiety and muscle tension reactivity in the regular user. Caffeine administration appears to relieve the anxiety and decrease reaction time. Since these effects are apparent after a relatively short abstinence, they may contribute to the maintenance of regular caffeine use. This hypothesis is supported by Mikkelsen's report that excessive caffeine consumption occurred with psychiatric patients at a time when they experienced anxiety from sources unrelated to caffeine use (*4*).

Although the physiological correlates of caffeine tolerance and withdrawal have not been identified, several caffeine-related changes may underlie the anxiety and muscular effects reported here. Caffeine produces contraction of human striated muscle by facilitating calcium transport, alters release and metabolism of monoamines, and inhibits phosphodiesterase, which is involved in the metabolism of cyclic nucleotides (*10*).

Progress has been made in the characterization of a caffeine withdrawal syndrome (*11*). We suggest that anxiety and muscle tension be added to the list of caffeine withdrawal symptoms, which already includes headache, irritability, drowsiness, and lethargy. Perhaps caffeine withdrawal deserves diagnostic status similar to that of caffeine toxicity—caffeinism (*12*).

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6. In addition to informed consent, subjects were screened for circulatory, thyroid, gastric, or other problems that might be affected by caffeine or citric acid. Information about recent medication and tobacco use were solicited in the initial questionnaire. These factors were comparable for the caffeine and noncaffeine groups. Only four subjects in each group smoked more than two cigarettes per day, precluding evaluation of smoking habits as a correlate of caffeine use, anxiety, or muscle tension. One smoker was in the high-intake caffeine group and three were in the low-intake group. The noncaffeine group had three smokers in the high intake subgroup and one in the low. Although no subjects withdrew from the study, their right to withdraw was stated when they initially volunteered and restated when they came to the laboratory. At the completion of the experiment, all subjects were informed of their actual treatment and of the major results of the study.
7. Grapefruit juice was selected as the vehicle because of its acidic and slightly bitter taste, which, to our perception, completely masked the taste of the caffeine citrate and citric acid. At the end of testing, each subject was asked whether his or her grapefruit juice had caffeine in it. Only 4 of the 19 caffeine subjects and 8 of the 17 placebo subjects correctly identified their conditions.
8. Caffeine concentrations reported in *The Medical Letter* [**19**, 65 (1977)] were used to estimate caffeine consumption in brewed coffee (125 mg per cup), instant coffee (70 mg per cup), tea (70 mg per cup), and soft drinks containing caffeine (25 mg per cup). These values are somewhat arbitrary in that other sources (*4*) report higher concentrations of caffeine per cup of coffee. Our estimates are conservative and may underestimate actual consumption. However, we believe that the relative differences between individual subjects are accurate, because students at Centre College are required to participate in the food services, and therefore have a common source of coffee, tea, and cola beverages. For this reason we have considerable confidence in our correlational analyses.
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Juvenile Hormone Induction of Biting Behavior in *Culex* Mosquitoes

Abstract. *Juvenile hormone deprivation caused by surgical removal of corpora allata shortly after adult emergence blocked the initiation of biting behavior in* Culex pipiens *and* Culex quinquefasciatus. *Reimplantation of corpora allata or injection of a synthetic juvenile hormone (JH-I) corrected the juvenile hormone deficiency and restored biting behavior. Ovariectomy experiments demonstrated that this behavioral effect of juvenile hormone was independent of ovarian involvement.*

In terms of disease transmission, mosquitoes are among the world's most important blood-feeding insects. Pathogens and parasites transmitted by the bites of mosquitoes cause encephalitis, dengue, filariasis, malaria, yellow fever, and a number of other well-known diseases. Yet, despite the epidemiological significance of blood feeding, little information is available on the events that initiate mosquito biting behavior.

Adult mosquitoes feed readily on sugar soon after they emerge from the aquatic pupal stage. There is usually a 2- to 3-day interval, however, before the female initiates biting behavior. During this interval, mosquitoes initiate sexual behavior and begin to develop ovarian follicles. Since these last events are induced by juvenile hormone (JH) (*1*), several investigators have speculated that biting behavior is also hormonally induced (*2, 3*). To test this hypothesis, we investigated the effect of JH on the biting behavior of the mosquitoes *Culex pipiens* and *Culex quinquefasciatus*. Our results provide experimental evidence for the hormonal induction of biting behavior in a blood-feeding insect.

Mosquitoes were reared and maintained in a daily photoperiod of 14 hours of light and 10 hours of darkness at 26°C (*4*). Larvae were fed a diet described previously (*5*). The procedures we used for surgical removal of the corpora allata (allatectomy), the ovaries (ovariectomy), and hormone injection have also been described (*6, 7*).

Earlier studies on *C. pipiens* (*8*) established that allatectomy of 1-hour-old adults created a JH deficiency that prevented the development of resting-stage ovarian follicles, an event that precedes egg maturation in mosquitoes (*9*). We therefore subjected *C. pipiens* and *C. quinquefasciatus* to allatectomy 1 hour after they emerged to determine whether JH deprivation would prevent the initiation of biting behavior. Mosquitoes subjected to sham operations or no operations were used as controls. Sham operations involved opening and then resealing the neck membrane without removing the corpora allata.

The mosquitoes were placed in Formica and Plexiglas cages (26 by 18 by 18

Table 1. Biting behavior in *C. pipiens* and *C. quinquefasciatus* subjected to allatectomy 1 hour after emergence. The number of mosquitoes biting is shown over the number tested.

Group	Day 7*				Day 14				Total†			
	C. pipiens		*C. quin.*		*C. pipiens*		*C. quin.*		*C. pipiens*		*C. quin.*	
	Number biting	Percentage	Number biting	Percentage	Number biting	Percentage	Number biting	Percentage	Number biting	Percentage	Number biting	Percentage
Allatectomized	0/31	0	0/25	0	0/29	0	0/18	0	0/31	0	0/25	0
Control (sham operation)	22/37	59	8/20	40	3/14	21	4/9	44	25/37	68	12/20	60
Control (no operation)	28/39	72	10/27	37	2/10	20	10/15	67	30/39	77	20/27	74

*Mosquitoes feeding on day 7 were excluded from biting tests on day 14. Discrepancies in numbers of mosquitoes tested on day 14 represent mortality in the test populations between days 7 and 14. †Percentage of allatectomized mosquitoes showing biting behavior was significantly different from that of both control groups at $P = .01$ when calculated as the standard error of the percentage difference (*16*).

Table 2. Biting behavior in allatectomized (−CA) *C. pipiens* and *C. quinquefasciatus* induced by CA implantation (+CA) or by injection of 5 μg of JH-I (+JH). The mosquitoes were allatectomized 1 hour after emergence and were injected with JH in 0.25 μl of olive oil or with 0.25 μl of olive oil alone 1 or 2 days after emergence. The number and percentage biting were determined 7 days after emergence.

Treatment	*C. pipiens*		*C. quinquefasciatus*	
	Number biting	Percentage*	Number biting	Percentage*
−CA, +CA	11/16	69	24/46	52
−CA + fat body	0/17	0	0/17	0
Control (no operation)	23/32	72	17/29	59
−CA, +JH	9/17	53	12/19	63
−CA + olive oil	0/23	0	0/18	0
Control (no operation) + olive oil	33/36	92	27/29	93

*Percentage biting of allatectomized controls implanted with fat body or injected with olive oil was significantly different from the corresponding test groups when calculated as the standard error of the percentage difference.

cm) in which a solution of 10 percent sucrose was available. Biting behavior was determined on nights 7 and 14 by allowing the mosquitoes access to a chick confined in a test tube basket or nylon stocking. Mosquitoes that failed to bite on the night of day 7, as evidenced by the visual absence of blood in the midgut, were tested again on day 14.

Table 1 shows that the allatectomized mosquitoes failed to bite, whereas high percentages of both control groups (mosquitoes with sham operations or no operations) showed biting behavior. Thus allatectomy not only blocked the post-emergence induction of biting behavior, but prevented the initiation of biting for the 2-week duration of the experiment. Since corpora allata are the source of mosquito JH (*1*), these results suggested that JH deprivation blocked biting behavior. This hypothesis was supported by earlier work demonstrating that allatectomy 3 to 5 days after emergence (that is, after JH release) allowed normal biting in *C. pipiens* (*10*).

In a second experiment we subjected 1-hour-old females to allatectomy and immediately reimplanted the corpora allata in the neck. As controls for this experiment we used both allatectomized females in which we implanted pieces of fat body instead of corpora allata and females that received no operations. Another group of allatectomized mosquitoes received on day 2 an injection of either 5 μg of JH-I (*11*) in 0.25 μl of olive oil or 0.25 μl of olive oil alone. Controls for this group received an injection of 0.25 μl of olive oil but no operation.

Table 2 shows that biting behavior was restored both by reimplantation of corpora allata and by injection of JH-I. We therefore concluded that biting was initiated in response to the secretion of JH from the corpora allata.

Since JH initiates the development of resting-stage follicles, the results of our experiments did not rule out the possibility that biting behavior might be induced by nervous or hormonal stimuli from the ovaries. We therefore conducted another experiment in which mosquitoes were ovariectomized 1 hour after emergence. These mosquitoes were tested for their ability to initiate biting in the same manner as were the allatectomized mosquitoes used in previous experiments. Control mosquitoes received no operations. The results of this experiment showed no differences in biting behavior between ovariectomized and control mosquitoes. The percentages biting 7 days after emergence were as follows: ovariectomized, 71 percent of both *C. pipiens* and *quinquefasciatus*; controls, 87 percent of *C. pipiens* and 74 percent of *C. quinquefasciatus*. Ovarian involvement was thus ruled out.

That JH induces biting behavior in two species of mosquitoes probably explains the absence of blood-feeding activity in diapausing *Culex* frequently observed by other investigators (*12*). In *C. pipiens*, diapause is induced in the fall by seasonal changes in photoperiod and temperature that suppress corpus allatum function in newly emerged adults (*8*). The consequent JH deficiency apparently blocks the induction of biting behavior as well as follicular development and thus restricts the mosquito to a diet of plant juices. This well-known dietary modification prevents reproduction in the fall and results in the accumulation of extensive fat reserves prior to low temperature-enforced hibernation.

Fall broods of *C. pipiens* occasionally bite in response to prolonged periods of warm temperature. Under these circumstances, females sometimes feed on blood without developing eggs, a phenomenon known as gonotrophic dissociation (*13, 14*). Since these "dissociating" females develop resting-stage follicles concurrent with the initiation of biting behavior (*14*), they must also release JH. Thus, gonotrophic dissociation in *Culex* appears to be independent of the JH requirement for biting behavior.

It is not clear to what extent JH initiates biting behavior in other blood-feeding insects. Our results with *C. quinquefasciatus* show that this JH effect is not limited to mosquitoes that undergo diapause. However, evidence that *Aedes* mosquitoes bite despite allatectomy (*6*) suggests that JH induction of biting behavior is not ubiquitous among mosquitoes. Nevertheless, since several mosquitoes that overwinter as adults in the genera *Culex*, *Anopheles*, and *Culiseta* show the same modification in feeding behavior as diapausing *C. pipiens*, JH may initiate biting behavior in many of these insects.

The possibility that JH initiates biting behavior in *Anopheles freeborni* has already been suggested by Case *et al.* (*3*). They found that the JH mimic Methoprene stimulated both biting behavior and diapause termination. However, a second JH mimic (6,7-epoxygeranyl-4-ethylphenyl ether) failed to elicit biting despite diapause termination. Case *et al.* postulated that a natural JH might initiate biting in *Anopheles freeborni*, and that the discrepancy in results was due to differences in molecular structure between the mimics tested and natural JH's.

Recent studies suggest that JH is not the only hormone involved in the regulation of mosquito biting behavior. In *Aedes aegypti* and *Anopheles freeborni*, ovaries with developing eggs secrete a hormone that suppresses host-seeking or biting behavior between gonotrophic cycles (*15*). Whether this ovarian hormone influences JH synthesis to prevent biting during egg development is unknown.

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5. Mosquito larvae (100 per 350 ml of tap water) were reared in covered plastic pans (27 by 19 by 6 cm) on a diet consisting of equal parts Brewer's yeast, lactalbumin, and finely ground laboratory animal chow. Daily rations varied with larval stage; newly hatched (day 0) and day 1, 150 mg; day 2 and day 3, 250 mg; day 4, 450 mg; and day 5, 250 mg.
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Have Tar and Nicotine Yields of Cigarettes Changed?

Abstract. *In official assays of the tar and nicotine yields of 12 popular brands of cigarettes, smoking machines took fewer puffs, on the average, in 1974 than in 1969. The decline in puffs appears to have been a major cause of the reported reductions in tar and nicotine yields during this period.*

Since 1967 in the United States and 1969 in Canada, the governments have sponsored regular assays of the "tar" and nicotine yield of cigarettes by means of smoking machines (*1*). These assays show that the tar deliveries of most cigarette brands have declined in the last 10 years (*2*). The value of the published figures has been criticized repeatedly, however, on the grounds that smokers can compensate for reduced deliveries by altering the way they smoke, for instance by taking more or larger puffs (*3*). Clearly, the advantages of switching to "milder" cigarettes depend on the degree to which smoking behavior remains unchanged. Similarly, fair comparisons of tar and nicotine deliveries require the behavior of the smoking machines to be held constant. We believe that a loophole exists in the standard smoking-machine procedure in that it does not specify the number of puffs to be taken. The number of puffs taken per cigarette for some brands declined significantly from 1969 to 1974, and we believe that this change has contributed to the reported reductions in their tar and nicotine content.

Many people may assume that, puff for puff, newer versions of popular brands (*4*) have been becoming weaker in tar and nicotine. The standard procedure, however, fixes neither the number of puffs taken on different brands during the same test nor the number taken on the same brand in subsequent tests. The procedure prescribes that a smoking machine (essentially a motorized syringe) take a 2-second 35-ml puff once each minute until a fixed butt length is reached (*5*). Number of puffs is determined, then, by the burn-time of the cigarette. Burn-time can be influenced, for example, by the porosity of the cigarette paper or the amount of tobacco in the cigarette.

The Federal Trade Commission (FTC) laboratory has not saved records of the number of puffs taken per cigarette in its tests (*6*), but in Canada such information has been kept, although it has never been studied systematically or published. We report here an analysis of puff data for 12 of the best-selling Canadian filter cigarettes, which accounted for 60 percent of the cigarette market in 1970 and 70 percent in 1974 (*7*). Our analysis was limited to the 11 Canadian surveys between 1969 and 1974, in which assays were done on the same machine and with the same analytical procedures (*2, 8*).

For the 12 brands as a group, decreases in tar (actually "wet tar") (*2*) were strongly associated with decreases in the number of puffs taken by the smoking machine ($r = .97$, $P < .01$, d.f. = 4) (Fig. 1). There is a similar association between puffs and tar for each

Table 1. Comparison of the yields and weights (mean ± standard deviation) of 12 popular brands of Canadian cigarettes in survey 1 (1969) and survey 11 (1974). Paired *t*-tests (two-tailed) are used.

Item	Survey 1 (1969)	Survey 11 (1974)	Range of differences
Tar (mg)	21.8 ± 1.98	18.6 ± 2.24*	1.3 to 6.9
Nicotine (mg)	1.31 ± 0.14	1.15 ± 0.14†	−.07 to .49
Weight (g)	1.12 ± 0.08	1.06 ± 0.08*	.03 to .11
Puffs	9.8 ± 1.1	8.8 ± 0.98*	.4 to 1.9
Tar per puff	2.24 ± 0.21	2.12 ± 0.22†	−.04 to .30
Nicotine per puff	0.135 ± 0.011	0.131 ± 0.012‡	−.013 to .025

*$P < .002$. †$P < .01$. ‡Not significant.

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