

LETHAL EFFECTS OF SYNTHETIC JUVENILE HORMONE ON THE HUMAN BODY LOUSE*

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The effectiveness of synthetic juvenile hormone in the control of noxious insects and arthropod vectors of disease has already been documented in the case of the yellow-fever mosquito, *Aedes aegypti*.¹ The present study reports the lethal action of this same synthetic juvenile hormone on *Pediculus humanus* var. *corporis*, the vector of epidemic typhus, trench fever, and epidemic relapsing fever.

Materials and Methods.—Lice were from a stock colony adapted to feed on rabbits. The colony was kept on woolen pads, fed to repletion once a day, and at other times stored at 30–32°C and a relative humidity of 50–60 per cent.

Synthetic juvenile hormone (SJH) was prepared as described by Law *et al.*² by bubbling hydrogen chloride gas through an ice-cold solution of farnesenic acid (Frinton Laboratories, South Vineland, New Jersey) dissolved in 100 volumes of absolute ethanol. The flow of gas was continued for five minutes to give a 9.4 per cent increase in weight. The flask was stoppered and stored in an ice bath in the dark for four hours. It was then warmed to room temperature and stored in the dark for an additional 19 hours. The reaction mixture was rinsed with petroleum ether into a separatory funnel and washed three times with an excess of water. The ethereal phase was collected and the solvent removed on a rotary evaporator at room temperature. The resultant golden oil was rinsed with 95 per cent ethanol into a separatory funnel and water added to incipient cloudiness. The solution was neutralized with 0.1 *M* sodium hydroxide to a phenolphthalein end-point and the ethereal phase collected and reduced to dryness on a rotary evaporator. The resultant golden oil, which was used without any further purification, is known to contain six materials with high juvenile hormone activity. For convenience of expression in the present report, this mixture of materials is called “synthetic juvenile hormone.”

Exposure of lice and lice eggs to SJH: Adult lice were placed on 3- × 3-cm woolen pads impregnated with known concentrations of SJH and eggs were deposited on the pads. In control experiments lice were placed and eggs were laid on pads impregnated with a corresponding amount of peanut oil. Each pad was impregnated with 1 ml of a solution of SJH or peanut oil in absolute methanol; the solvent was evaporated before use.

The number of eggs on each pad was counted with the aid of a dissecting microscope. Empty or collapsed eggs were discarded. To facilitate counting, a piece of glass marked into a grid with a diamond pencil was placed over the pad. The grid was rinsed in acetone and in 95 per cent ethanol to prevent contamination of subsequent pads.

Experimental and control lice were fed in separate compartments of an arena on the shaved belly of a rabbit. The shaved skin was washed with 70 per cent ethanol before and after feeding and the arena with acetone after each use.

Preliminary tests showed that SJH at a concentration of 10 mg per pad was lethal for adult lice within two hours. However, concentrations of from 1 to 4 mg per pad were tolerated without any apparent immediate injury to the lice.

Nymphal and adult lice moved freely among the fibers of the woolen pad and were therefore in continuous contact with the impregnated surface. This was not true of all eggs, for some were nestled in the fabric while others were laid at various angles and frequently were attached to the pad by a single wool fiber. In several experiments attempts were made to reduce the resulting variation in dosage by replacing the woolen pads with nylon mesh. A calculated amount of SJH was pipetted onto the mesh and the solvent rapidly evaporated in a stream of warm air.

Experimental Results.—Effects of continuous exposure of eggs to SJH: Three representative experiments document the effectiveness of SJH in blocking embryonic development of treated eggs. In experiment A a group of adult lice (28 females and 12 males) was placed for 24 hours on a woolen pad impregnated with 1 mg SJH. They were then removed. A control group was similarly exposed to a pad treated with 1 mg peanut oil. Eggs laid on both pads were counted, as were emerging nymphs.

In experiment B, 20 females and 10 males were placed for 24 hours on nylon mesh coated with 0.2 mg SJH. The control group of 18 females and 8 males was placed on nylon mesh coated with a corresponding amount of peanut oil. Again, eggs and nymphs were counted.

In another test (experiment C) 15 females and 5 males were placed on a woolen pad impregnated with 1 mg SJH. At 24-hour intervals for a total of seven days the lice were transferred to fresh pads impregnated with 1 mg SJH. A control group was handled in exactly the same manner except that the pads were impregnated with 1 mg peanut oil. Counts were made of the number of eggs laid on all pads and also of the number of nymphs which hatched.

Results from the three experiments are summarized in Table 1, where two phenomena can be observed. First, continuous exposure of eggs to SJH severely reduced the number which hatched, and second, the only eggs which hatched were derived from females with less than one day of exposure to the hormone. In experiment A, 24 per cent of the eggs in the experimental group and 82 per cent of the controls hatched. This difference is highly significant ($p = <0.01$) though not as great as in experiments B and C, where 0 and 7 per cent, respectively, of the

TABLE 1
EFFECT OF SYNTHETIC JUVENILE HORMONE ON EGGS LAID BY FEMALES WHICH WERE EXPOSED TO HORMONE FOR VARYING PERIODS OF TIME

Eggs were continuously exposed to hormone.

Expt.	Treatment pad	Hours females treated before eggs laid	Eggs Continuously Exposed to			
			SJH		Peanut Oil (Control)	
			No. hatched/total	Hatched, %	No. hatched/total	Hatched, %
A	Wool*	0-24	18/76	24	66/78	82
B	Nylon†	0-24	0/44	0	28/32	88
C	Wool*	0-24	3/42	7	37/44	84
C	Wool*	24-144	0/272	0	191/226	85

* Lice were placed and eggs were laid on 3- × 3-cm woolen pads impregnated with 1 mg SJH. Control pads were impregnated with 1 mg peanut oil.

† The treatment pad was 3- × 3-cm nylon mesh coated with 0.2 mg SJH. Control mesh was coated with 0.2 mg peanut oil.

treated eggs hatched. Of the 272 treated eggs laid by females having more than 24 hours of exposure to the hormone, none hatched.

Effects of SJH on eggs laid by pretreated females: In experiment D adults (23 females and 12 males) were placed for 24 hours on a woolen pad impregnated with 1 mg SJH. After this exposure period the lice were transferred to an untreated pad where the females laid eggs. At daily intervals thereafter, the adults were transferred to fresh untreated pads. The control group consisted of 21 females and 13 males exposed for 24 hours to a pad impregnated with 1 mg of peanut oil. The eggs and hatching nymphs were counted on all pads.

The relevant data are summarized in Table 2, where it can be seen that hatching was significantly suppressed among eggs laid on the untreated pad during the first 24 hours following treatment of the females with hormone. After one day in the absence of SJH the effect completely vanished and the pretreated females laid eggs which hatched in the normal way.

Effects of SJH on postembryonic development and metamorphosis: Approximately 200 freshly hatched nymphs were fed and immediately transferred to a woolen pad impregnated with 2 mg SJH. A control group was treated in like manner and placed on a pad impregnated with 2 mg peanut oil. Both groups were fed and observed daily for 28 days.

All individuals showed normal development until the end of the third instar stage, when metamorphosis accompanied by sexual maturation normally occurs. At this point, the experimental and control groups showed a spectacular difference in further development. The control group metamorphosed, mated, deposited eggs, and began a second generation which repeated the process to form a third generation during the 28-day period of the experiment. The normal multiplication of numbers required the addition of two more pads impregnated with peanut oil. By contrast, none of the nymphs exposed to SJH was able to complete development. Many of them underwent one or more additional nymphal molts and grew to considerable size. Mortality was high and no eggs were deposited. Finally, when the experiment was terminated on the 28th day, all that remained were ten giant adultoid lice (see Fig. 1).

Summary.—The present study demonstrates that synthetic juvenile hormone is an effective insecticide and ovicide for human body lice. The ovicidal action is similar to that described by Sláma and Williams³ for hemipteran eggs and by Riddiford and Williams⁴ for the eggs of silkworms. As in the studies cited, we also find that the synthetic hormone is fully effective in blocking metamorphosis and sexual maturation when brought into contact with the unbroken skin of nymphal lice.

TABLE 2
HATCHING OF UNTREATED EGGS DERIVED FROM FEMALE LICE PREVIOUSLY
EXPOSED TO SYNTHETIC JUVENILE HORMONE FOR 24 HOURS

Time eggs laid after exposure of females*	Eggs Derived from Females Previously Exposed* to				CR†	p
	SJH		Peanut Oil (Control)			
	No. hatched/total	Hatched, %	No. hatched/total	Hatched, %		
0-24 hr	39/84	46	76/79	96	7.0	<0.01
24-48 hr	44/53	83	51/58	88	0.7	>0.05

* Lice were treated by maintaining them for 24 hr on 3- × 3-cm woolen pads impregnated with 1 mg of SJH. Control lice were placed on pads impregnated with 1 mg of peanut oil.

† Critical ratio: where CR = 1.96, p = 0.5; where CR = 2.58, p = 0.01.

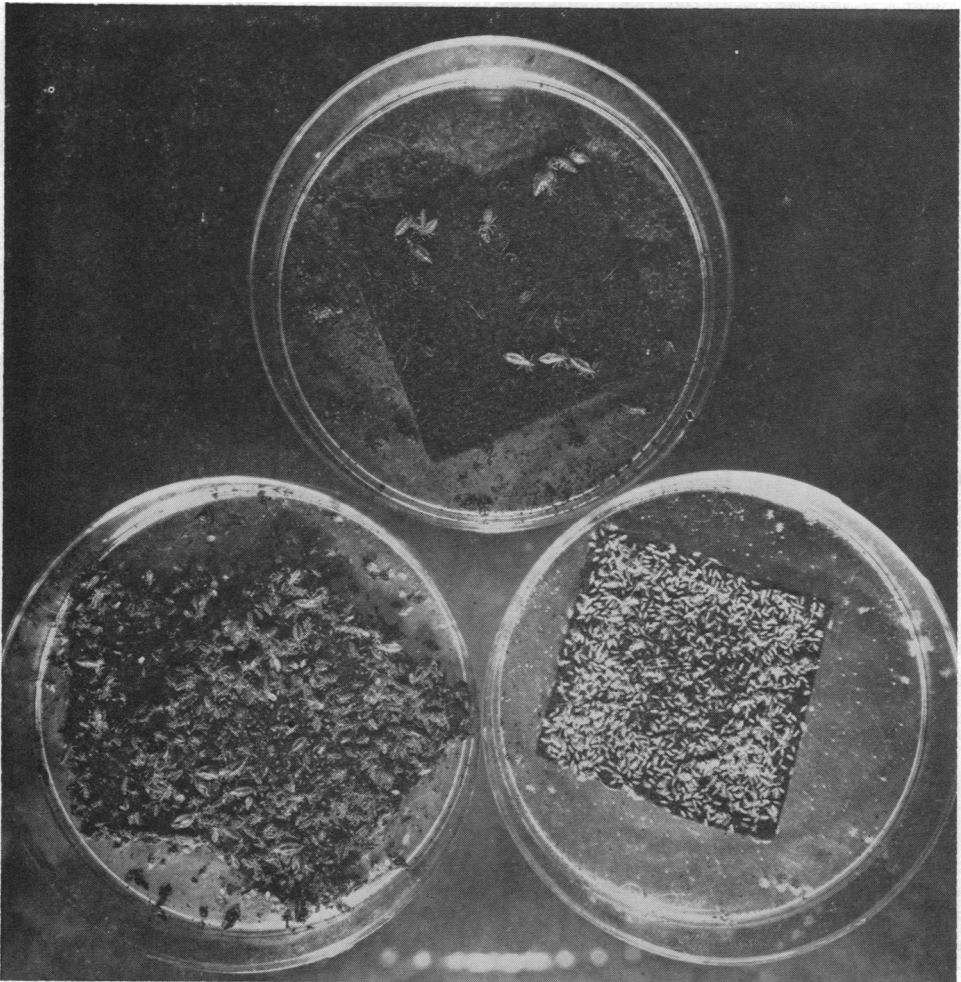


FIG. 1.—Effect of synthetic juvenile hormone on metamorphosis of lice. *Upper*: Only 10 adultoid forms remain from 200 lice maintained on a woolen pad impregnated with 2 mg SJH since their emergence from eggs 28 days previously. The nymphal lice could not metamorphose into sexually mature adults. *Lower*: Colony reared from 200 nymphs maintained on control pads impregnated with peanut oil. Lice now in their third generation. Note abundance of eggs, nymphs, and adults.

These findings are therefore of special interest because in many areas of the world the human body louse—vector of epidemic typhus, trench fever, and epidemic relapsing fever—has become resistant to virtually all presently known insecticides.

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¹ Spielman, A., and C. M. Williams, *Science*, **154**, 1043 (1966).

² Law, J. H., C. Yuan, and C. M. Williams, these PROCEEDINGS, **55**, 576 (1966).

³ Sláma, K., and C. M. Williams, *Nature*, **210**, 329 (1966).

⁴ Riddiford, L. M., and C. M. Williams, these PROCEEDINGS, **57**, 595 (1967).