meability observed in these cells. These latter findings complement research on erythrocyte membranes which has shown that salicylate increases cation permeability and decreases anion permeability (*7*). The membrane hyperpolarization and decrease in total membrane resistance of a particular neuron in the presence of salicylate would serve to decrease both the output from and the input to such a cell (*8*).

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4. This report is based on observations from 30 experiments on the cells G-R, M-R, G-L, M-LD, identified by H. Levitan, L. Tauc, J. P. Segundo [*J. Gen. Physiol.* **55**, 484 (1970)].
5. External concentrations of potassium were changed isosmotically by substituting NaCl for KCl. In saline with a low concentration of Na^+, 482 m*M* NaCl was replaced by 241 m*M* $MgCl_2$ and 234 m*M* mannitol, giving a solution isotonic with normal physiological saline but containing 10 m*M* Na^+ with a constant concentration of Cl^-. In saline with a low concentration of Cl^- NaCl was replaced by Na_2SO_4 so that the final concentration of Na^+ remained constant while that of Cl^- decreased to 70 mmole/liter. Mannitol (400 mmole/liter) was added to bring the osmolarity to 1100 milliosmoles per liter.
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8. On occasion we impaled cells whose resting membrane potential, input resistance, and sensitivity to changes in $[K^+]_o$ were significantly less than normal, which suggested injury. After a brief exposure to relatively small concentrations of salicylate (≤ 3 mmole/liter), the membrane properties of the cells were markedly changed: the membrane potential was greater than that before application of salicylate, whereas the resistance had increased relative to initial values, and the slope of the curve of membrane potential as a function of log $[K^+]_o$ was greater than that observed initially. These new values were maintained thereafter. It would appear that salicylate had a salutory effect on these neurons.

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Ovarian Maturation in Stable Flies: Inhibition by 20-Hydroxyecdysone

Abstract. *The steroid 20-hydroxyecdysone when given by mouth inhibits ovarian maturation in the stable fly,* Stomoxys calcitrans *(L.), by preventing lipid synthesis necessary for vitellogenesis in the developing oocyte.*

We report here that 20-hydroxyecdysone when ingested by the stable fly prevents vitellogenesis in developing oocytes. These results indicate that the labeled messenger RNA passes from the nucleus to the nurse cell cytoplasm but that the lipid material necessary for vitellogenesis is not synthesized in those flies treated with 20-hydroxyecdysone. This steroid (*1*) and related 6-keto-steroids inhibit ovarian development when ingested by the stable fly [*Stomoxys calcitrans* (L.)] (*2*), the house fly (*Musca domestica* L.), the confused flour beetle (*Tribolium confusum* Jacquelin duVal) (*3*), and the boll weevil (*Anthonomus grandis* Boheman) (*4*). Several physiological and biochemical processes in insects have been suggested as being influenced by the insect molting hormone, α-ecdysone (*5*), and Neufeld *et al.* (*6*) showed that protein synthesis increased in the body wall and fat body of *Calliphora* larvae 4 hours after injection of 20-hydroxyecdysone; however, the role of 20-hydroxyecdysone in the inhibition of ovarian maturation in insects is still undetermined.

Young female stable flies were permanently sterile after having ingested a 0.1 percent solution of 20-hydroxyecdysone in fresh citrated beef blood for three consecutive days (*2*). To elucidate the biological activity of 20-hydroxyecdysone, we fed groups of similar flies from the laboratory colony a similar solution for four consecutive days (first day of feeding 12 hours after eclosion). For 4 days, beginning 24 hours after feeding started, ten flies were removed and each was injected with 1 μl of an aqueous solution of tritiated uridine (1.0 mc/ml, 5.0 c/mmole) (*7*). The ovaries were dissected 1, 6, and 24 hours after injection; fixed in Carnoy, Bouin, or formalin fixative; and sectioned at 7 μ. After fixation and clearance of the tissue on slides, the slides were dipped in nuclear track emulsion NTB-2 and held in lightproof boxes for 1 or 2 weeks before development in Dektol (*7*). The tissue was then stained with methylene blue. The same procedures were used with flies of the same ages divided into three control

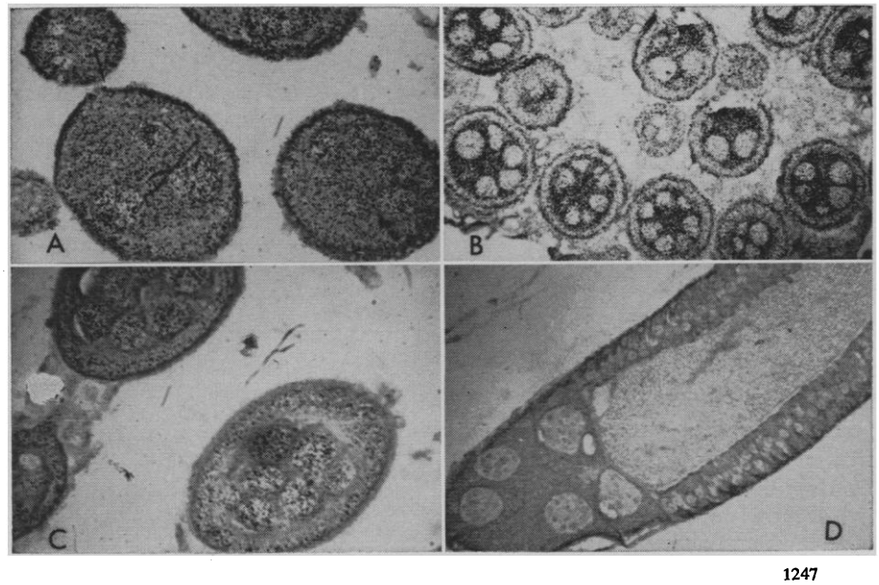

Fig. 1. (A) Day 1, 1 hour after injection with [^{3}H]uridine; the labeling in the control (no steroid) is in the cytoplasm and nucleus of the nurse cells (× 40). (B) Day 3, 6 hours after injection with [^{3}H]uridine; the labeling is in the cytoplasm of the nurse cells of the fly treated with 20-hydroxyecdysone (× 25). (C) Day 4, 1 hour after injection of [^{3}H]uridine; the labeling of the treated fly is in the nucleus of the nurse cells (× 40). (D) Untreated control on day 4 dissected 1 hour after injection of other flies with [^{3}H]uridine (× 25).

groups: one fed the steroid in citrated beef blood but not injected with tritium; one fed citrated beef blood without steroid and injected with tritium; and one fed citrated beef blood without steroid and not injected with tritium. The staging characteristics of oogenesis used in the analysis were based on those of Cummings and King (*8*).

In the day 1 labeled controls, the tritiated uridine was found in both the nucleus and cytoplasm of the nurse cells 1 hour after injection, but 6 and 24 hours after injection, it was found in the cytoplasm of the nurse cells. The pattern was similar at day 2. However, on days 3 and 4, labeling 1 hour after injection was primarily in the nucleus of the nurse cells; 6 and 24 hours after injection it was primarily in the cytoplasm, except that on day 4 no samples were taken 24 hours after injection. In flies fed 20-hydroxyecdysone, the labeling was like that in the controls except that on day 4 at 6 hours after injection the labeling was in the nucleus instead of the cytoplasm of the nurse cells. Throughout the experiment, the follicular cells of both the controls and the flies fed steroid were labeled similarly. No noticeable concentration of label was found in the yolky ooplasm of the controls.

The follicles of the treated and untreated flies developed similarly through stages 2 to 6 since the RNA was labeled first in the nucleus and later in the cytoplasm of the nurse cells. However, after day 2, the follicles in flies fed 20-hydroxyecdysone showed no further morphological changes in size or development and remained static in stage 6 (Fig. 1). In contrast, the oocyte within the ovarian follicle of the controls changed significantly from a spherical shape (follicle stage 6) to an elongated shape, which indicated that protein was synthesized in the nurse cell cytoplasm for vitellogenesis. The nurse cells degenerated, and the oocytes reached their maximum volume at day 4 of the control. The beginning development of the second follicular group in the ovaries was also observed at this time. This sequence was prevented in those flies treated with the 20-hydroxyecdysone.

The 20-hydroxyecdysone thus prevented the synthesis of the lipid materials necessary for vitellogenesis and final egg maturation. The time of activity occurred after the previtellogenic development as the egg chambers of both the treated and untreated flies grew at similar rates during this period. The egg chamber in the treated flies then abruptly stopped growth and thereby rendered the females sterile.

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Chemical Methylation of Inorganic Mercury with Methylcobalamin, a Vitamin B_{12} Analog

Abstract. *Chemical methylation of mercuric chloride with methylcobalamin has been studied. Methylated mercury was detected by gas chromatography; and analysis of the products of the reaction by thin-layer chromatography revealed that the methylation proceeded at a remarkably high rate when methylcobalamin and inorganic mercury were mixed. Dimethylmercury was an initial product of the reaction.*

Although use of organomercurial pesticides has been banned and the release of methyl- and ethylmercury into rivers and bays is restricted in Japan, relatively little attention has been paid to the risks arising from inorganic mercury pollution.

Jensen and Jernelöv have found that inorganic mercury is converted into methylmercury by some microorganisms (*1*), and that the bottom sediments of a lake and of an aquarium, as well as the homogenate of rotting fish, were capable of methylating mercury (*2*). Using an isolated strain of methanogenic bacterium (MOH), Wood *et al.* studied mercury methylation in vitro and showed that, in the presence of the crude extract of this microorganism, the methyl moiety of methylcobalamin

Table 1. Thin-layer chromatography of the reaction mixture containing methylcobalamin and varying amounts of $HgCl_2$. Methylcobalamin (2 μmole) was subjected to reaction with 1, 2, and 10 μmole of mercuric chloride, respectively, at 37°C in 1 ml of 0.2*M* potassium phosphate buffer (*p*H 7.0) in the dark. After 30 minutes, 5 hours, or 24 hours, the reaction mixture was extracted with 200 μl of benzene, and a 60-μl portion was analyzed by silica gel thin-layer chromatography with the use of solvent 1. Mercury was detected by spraying 0.05 percent dithizon-chloroform solution. The extent of the coloration of mercury dithizonate is expressed by ++ (strong), + (positive), ± (faint), and − (not detectable); D, dimethylmercury (R_F, 0.58); M, methylmercuric chloride (R_F, 0.25).

Molar ratio ($HgCl_2$/CH_3-[Co])	Detection of mercury after a reaction time of					
	30 minutes		5 hours		24 hours	
	D	M	D	M	D	M
0.5	+	−	+	−	+	−
1	+	±	+	++	±	++
5	+	++	±	++	−	++