be supposed that these concentrations of DFP or Tabun are in excess, by orders of magnitude, of what would have caused complete inhibition of axonal acetylcholinesterase. However, the difficulties in attempting to measure unambiguously the degree of inhibition of acetylcholinesterase in intact tissue are considerable and have frequently been reviewed (*1*, *4*, *16*).

Neither a role for DFP-hydrolyzing enzyme in nerve function, if indeed any exists, nor a natural substrate for this enzyme can be inferred from these results. In a more immediately practical context, it seems reassuring that toxic organophosphorus compounds such as DFP and related nerve gases and insecticides may be detoxified by enzymes in the squid and possibly in other inhabitants of the ocean. Such reassurance must be tempered by the marked substrate specificity described here and indicated previously for other classes of organophosphates (*2*).

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Salicylate: Effect on Membrane Permeability of Molluscan Neurons

Abstract. *Identified cells in the buccal ganglion of the marine mollusk* Navanax inermis *were exposed to salicylate (1 to 30 millimoles per liter) for short periods. Salicylate increased the permeability to potassium and decreased the permeability to chloride in a reversible, dose-dependent manner, producing a concomitant increase in membrane potential and a decrease in membrane resistance. These events would reduce the output from, as well as the effectiveness of synaptic input to, a particular neuron.*

Although salicylates are used daily as analgesics and antipyretics, little research has been reported on their effect on single neurons (*1*). We report here the results of experiments on the effect of salicylates at the membrane of molluscan neurons.

The buccal ganglion from the marine mollusk *Navanax inermis* (*2*) was isolated, mounted in a 1-ml Lucite chamber and perfused with normal physiological saline (*3*) at room temperature (22° to 24°C). After opening the capsule enveloping the ganglion, we impaled identified cells (*4*) with double-barreled micropipettes filled with 3*M* KCl (2 to 10 megohm resistance). One barrel was used to record membrane potential while the other allowed passage of current across the membrane.

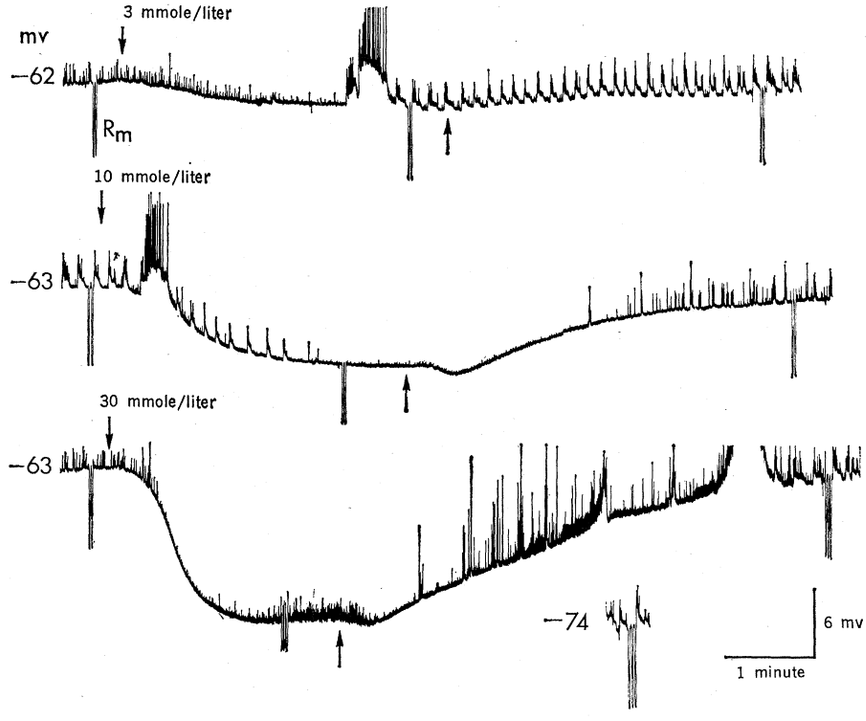

Fig. 1. Salicylate increases transmembrane potential and decreases membrane resistance. The results illustrated were obtained from cell M-R and are representative of all cells studied. The input resistance of the cell was obtained in normal physiological saline at a resting membrane potenital of −62 mv by passage of 4-na hyperpolarizing pulses 800 msec long (R_m) through a second intracellular electrode. Perfusion of the 1-ml chamber with 50 ml of physiological saline containing 3 m*M* sodium salicylate (downward arrow) caused a 2-mv hyperpolarization of the membrane potential with little change in resistance. Upon washing (upward arrow) with saline free of salicylate both the membrane potential and resistance returned to their control values. Subsequent tests with 10 m*M* and 30 m*M* salicylate produced greater hyperpolarizations (6 and 12 mv, respectively) and decreases in resistance (from 1.6 to 1.4 and 0.7 megohm, respectively). The upward deflections from the baseline are due to superimposed synaptic activity. The amplitude of these postsynaptic potentials is reduced in salicylate and readily recovers upon washing. (The record showing the recovery from 30 m*M* salicylate is interrupted briefly by a burst of synaptic activity which saturated the pen-recorder.) The membrane does not show rectifying properties over this range of potentials, and so in the absence of salicylate the resistance at −74 mv is the same as that at −63 mv (inset, lower right).

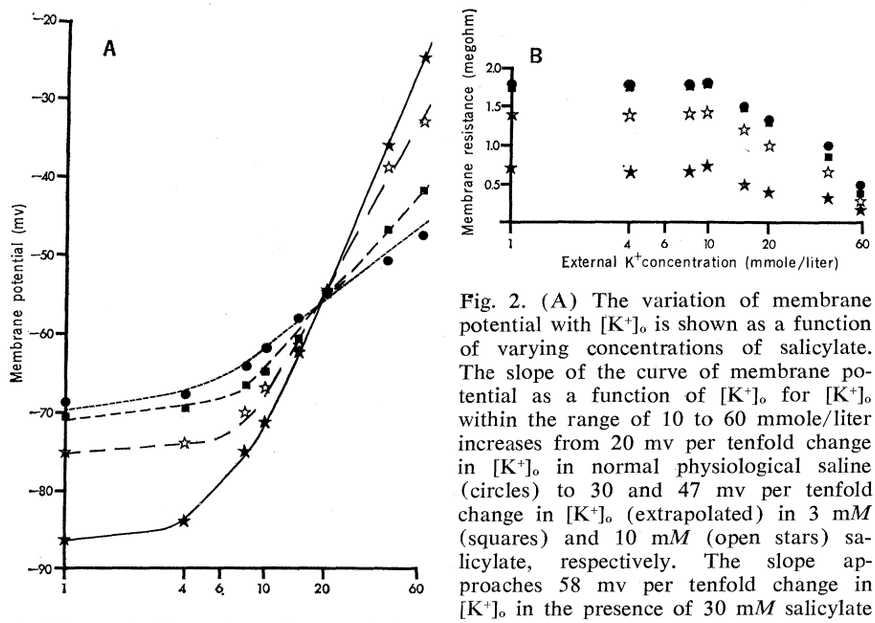

Fig. 2. (A) The variation of membrane potential with $[K^+]_o$ is shown as a function of varying concentrations of salicylate. The slope of the curve of membrane potential as a function of $[K^+]_o$ for $[K^+]_o$ within the range of 10 to 60 mmole/liter increases from 20 mv per tenfold change in $[K^+]_o$ in normal physiological saline (circles) to 30 and 47 mv per tenfold change in $[K^+]_o$ (extrapolated) in 3 m*M* (squares) and 10 m*M* (open stars) salicylate, respectively. The slope approaches 58 mv per tenfold change in $[K^+]_o$ in the presence of 30 m*M* salicylate (solid stars). The K^+ sensitivity of the membrane potential at $[K^+]_o$ values of 10 mmole/liter or less is also greatly enhanced by salicylate. (B) Total membrane resistance is proportionally reduced by increasing concentrations of salicylate at all values of $[K^+]_o$. The same symbols have been used in A and B to designate the different salicylate concentrations.

Electrical activity was amplified with standard techniques, displayed on an oscilloscope, and recorded on a pen-writer.

Cells were exposed to salicylate (1 to 30 mmole/liter) in the form of sodium salicylate dissolved in the physiological saline just before perfusion. In all cases salicylate caused an immediate, dose-dependent hyperpolarization of the resting membrane potential and a decrease in the cell's input resistance (Fig. 1), which were maintained as long as salicylate was present. Washing the cells with saline free of salicylate resulted in a rapid return of the membrane potential and resistance to control values. The decrease in resistance was due to the presence of salicylate rather than to a shift in the membrane potential itself since no comparable change in resistance was observed when the membrane was polarized to the same degree by application of transmembrane currents (Fig. 1, inset).

To elucidate the ionic mechanisms involved in salicylate's action, we changed the external concentrations of either K^+, Na^+, or Cl^- ($[K^+]_o$, $[Na^+]_o$, $[Cl^-]_o$) with and without salicylate present (*5*). The slope of the curve of membrane potential as a function of log $[K^+]_o$, for $[K^+]_o$ within the range of 8 to 100 mmole/liter, averaged 33 mv per tenfold change in $[K^+]_o$ (range 18 to 37 mv) in normal physiological saline (Fig. 2A). Salicylate increased this slope in a reversible, dose-dependent manner to a maximum 58 mv per tenfold change in $[K^+]_o$. The slope of the curve for $[K^+]_o$ within the range of 1 to 8 mmole/liter was also steepened by salicylate. The dose-dependent decrease in the cell's resistance was present over the entire range of $[K^+]_o$ studied (Fig. 2B).

Reducing $[Cl^-]_o$ from 572 to 70 mmole/liter caused a depolarization of the membrane potential with initiation of spike activity (Fig. 3). Salicylate attenuated this response in a reversible, dose-dependent manner. Figure 3 illustrates an attenuation of the response to a change in $[Cl^-]_o$ produced by the presence of 20 m*M* salicylate. These results indicate that salicylate reduces the cell's permeability to chloride, thus making the membrane potential less sensitive to changes in $[Cl^-]_o$. Salicylate did not alter the 1- to 2-mv hyperpolarization of the membrane potential observed upon reduction of $[Na^+]_o$ from 492 to 10 mmole/liter.

In summary, salicylate caused a hyperpolarization of the membrane potential and a decrease in total membrane resistance in all cells studied. Salicylate has a well-documented inhibitory effect on metabolic processes (*6*); however, this effect requires at least 30 minutes' exposure. Our results indicate that salicylate can alter the membrane potential and resistance within seconds, leading us to conclude that these events reflect changes in membrane permeability properties rather than alterations in metabolic processes. We propose that the changes in membrane potential and input resistance in the presence of salicylate result from the increase in K^+ permeability and decrease in Cl^- per-

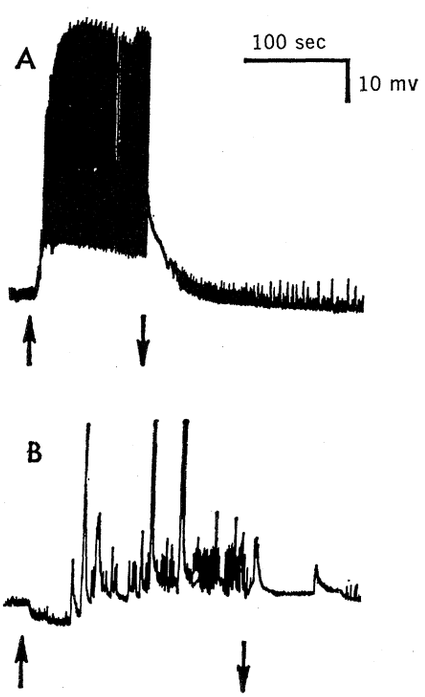

Fig. 3. Salicylate attenuates the response of the membrane potential to changes in $[Cl^-]_o$. The records displayed are from the cell M-R and are representative of all cells studied. In both A and B, the initial $[Cl^-]_o$ is 572 mmole/liter. The upward arrow indicates replacement of the Cl^- with SO_4^{2-} so that $[Cl^-]_o$ equals 70 mmole/liter. The downward arrow indicates rinsing with saline and return of $[Cl^-]_o$ to 572 mmole/liter. The control trace (A) shows that replacing most of the external chloride with SO_4 (upward arrow) results in a baseline depolarization of about 18 mv upon which is superimposed spike and synaptic activity. The membrane potential returns to its initial level (—59 mv) upon washing in physiological saline (downward arrow). Exposure to physiological saline containing 20 m*M* salicylate increases the membrane potential from —59 to —76 mv. Reducing the $[Cl^-]_o$ in the presence of salicylate now causes a much smaller depolarization of resting potential (about 2 mv). The initial, small hyperpolarization seen in this trace as $[Cl^-]_o$ is changed may be due to a junction potential or may reflect the membrane's permeability to SO_4.

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

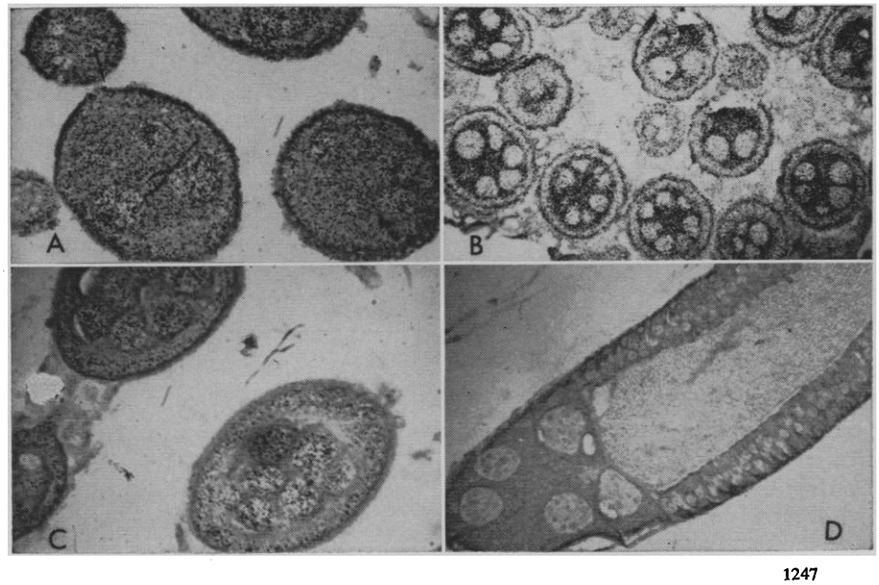

Fig. 1. (A) Day 1, 1 hour after injection with [^{3}H]uridine; the labeling in the control (no steroid) is in the cytoplasm and nucleus of the nurse cells (× 40). (B) Day 3, 6 hours after injection with [^{3}H]uridine; the labeling is in the cytoplasm of the nurse cells of the fly treated with 20-hydroxyecdysone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