The results in Table 3 show that, within as short a period as 70 minutes after administration of 0.5 mg of actinomycin per kilogram, there is a significant drop in the RNA/DNA ratio of the nucleus; a higher dose caused an even greater drop. In all experiments there was a positive correlation between the RNA/DNA ratio of the nucleus and its capacity to synthesize RNA, whether measured in vivo or in vitro. Others have shown that act/nomycin D blocks incorporation of labeled precursors into RNA (1); however, few have correlated the results of these studies with the RNA content of the nucleus. Revel and Hiatt (3) did report a decrease in RNA concentration in the nucleus following administration of actinomycin; however, the significance of their data is questionable since their values for the RNA/DNA ratios of both isolated nuclei and whole cells in normal rats are 2.0 to 2.5 times those of well-established values (16).

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Tetrodotoxin and Manganese Ion: Effects on Action Potential of the Frog Heart

Abstract. Tetrodotoxin, which effectively suppresses the permeability of various tissues to sodium ions, has no effect on the calcium action potential of the fiber of barnacle muscle, which potential is produced by increase in permeability to calcium ions. Manganese ions, however, suppress the action potential. When applied to fiber of frog cardiac ventricle, tetrodotoxin suppresses the rate of rise of the action potential without affecting the overshoot; the suppressive effect of manganese ion is mainly on the overshoot of the action potential. This suggests that, in the action potential of the cardiac ventricle of the frog, the plateau phase is related primarily to the increase in permeability of the membrane to calcium ions.

The puffer-fish toxin, tetrodotoxin, abolishes action potentials in various tissues such as fiber of frog skeletal muscle (1), giant axon of squid (2), lobster axon (3), and electroplaque of electric eel (4) at extremely low concentrations (10^{-8} to 10^{-7} g/ml). In such tissues the action potential is considered to result from increase in permeability of the membrane to sodium ions, this being followed by increased permeability to potassium ions. Tetrodotoxin suppresses the increased permeability to Na without affecting permeability to K (1-4).

The action potential of the fiber of barnacle muscle is produced by an increase in permeability of the membrane to calcium ions instead of to Na ions (5). This is followed by increased permeability to K, a process similar to that occurring in other tissues. Further-



Fig. 1. Effect of tetrodotoxin on resting and action potentials of cardiac ventricle fiber of the frog. A, Normal Ringer solution; B, tetrodotoxin added at 10^{-8} g/ml; C, tetrodotoxin added at 2.5×10^{-8} g/ml. Three records were obtained from different measurements on the same ventricle preparation: a, slow-sweep-speed recording of potential, showing the whole sequence of the action potential; b, fast-sweep-speed recording of potential, showing only the rising phase of action potential; c, the rate of rise of the potential (electrical differentiation of b, with time constant of 100 μ sec; increasing rate of rise, downward). The base line of c corresponds to the outside zero potential. The sweep speed of C is the same as that of A and B. This also applies to records in Fig. 2.



Fig. 2. Effect of MnCl₂ on resting and action potentials of cardiac ventricle fiber of the frog. A, Normal Ringer solution; B, MnCl₂ added at 4 mM; C, MnCl₂ added at 8 mM; D, MnCl₂ added at 10 mM. Four pairs of records were obtained from different measurements on the same ventricle preparation (see legend to Fig. 1).

Table 1. Effects of tetrodotoxin (with four frog ventricles) and manganese ion (with two ventricles) on the rate of rise of the initial phase and the amplitude of overshoot of the plateau phase; means \pm S.D. Numbers of measurements appear in parentheses.

Concen- tration	Overshoot (mv)	Rate of rise, maximum (volts per second)
Te	trodotoxin (g/)	ml)
0 (9)	43 ± 5	36 ± 15
1 x 10 ⁻⁸ (19)	45 ± 5	20 ± 7
5×10^{-8} (8)	39 ± 6	6 ± 1
Ma	nganese ion (m	i M)
0 (19)	45 ± 6	50 ± 13
2 (12)	33 ± 5	48 ± 16
4 (14)	32 ± 3	47 ± 17
8 (18)	30 ± 6	33 ± 14

more, it has been shown that even at high concentrations (4 \times 10⁻⁶ g/ml) tetrodotoxin has no effect on the action potential induced by Ca ions (Ca spike) in fibers of barnacle muscle (6). By contrast, the Ca spike is suppressed or abolished when manganese ions are added to the external solution (6); this agrees with the result of Fatt and Ginsborg (7). The effect of Mn^{++} becomes less marked with increasing external concentration of Ca, suggesting that Mn++ acts as a competitive inhibitor of the initiation of the Ca spike (6).

Brady and Woodbury (8) showed that the initial rapid rise of the action potential of the frog cardiac ventricle is due to increase in conductance by the membrane of Na ions. However, Coraboeuf and Otsuka (9) observed that, at the peak of the action potential, the membrane of the guinea pig ventricle did not behave as a sodium electrode; and the recent data of Orkand and Niedergerke (10) suggest that Ca-ion permeability also may be implicated in the plateau phase of action potentials of fibers of frog ventricle. Thus the action potential reached a peak in the plateau phase sometime after the initial rapid rise, and the maximum active membrane potential (overshoot) was essentially independent of the external Na-ion concentration over a considerable range when the Na ions were replaced by choline ions (10). If the initial rapidly rising phase of the spike potential in the fiber of frog ventricle were produced by increased permeability to Na ions, and if the plateau phase were related principally to permeability by Ca ions, the major effect of tetrodotoxin would be on the rate of rise of the spike poten-

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tial, and the effect of manganese ions would be mainly on the overshoot.

Resting and spike potentials were recorded intracellularly by means of micropipettes filled with 3M KCl and inserted into a strip of isolated frog ventricle some distance from the stimulating electrodes; stimuli were delivered at an interval longer than one minute.

Application of tetrodotoxin (1 \times 10^{-8} g/ml) decreased the maximum rate of rise of the initial phase to about 60 percent of the control value, but the amplitude of the overshoot of the plateau phase did not change at this concentration (Table 1). A higher concentration (5 \times 10⁻⁸ g/ml) decreased the maximum rate of rise to about 17 percent of the control, whereas the overshoot was lowered by only 10 percent (Table 1). Plotting the relation between the rate of rise and the amplitude of overshoot obtained in each measurement (instead of grouping and averaging the values according to concentration) showed that there was essentially no change in the amplitude of the overshoot unless the rate of rise became less than 5 volt/sec-that is, about 14 percent of the control. When the rate decreased to below 5 volt/sec, the overshoot often became smaller, and in extreme cases, a spike of short duration, without the subsequent plateau phase, was observed. Fig. 1 shows typical examples of the marked effect of tetrodotoxin on the rate of rise and its relative lack of effect on the amplitude of overshoot.

When $MnCl_2$ (2 to 4 mM) was added to the Ringer solution by replacing the osmotically equivalent NaCl (Ca concentration was kept constant at 1.8 mM throughout), there was no significant change in the rate of rise (Table 1). This fact, however, caused a characteristic change in the plateau potential (Fig. 2). The potential tended to rise more slowly after the initial rapid rise, the development of the plateau being delayed (Fig. 2B). At the same time, the potential became lower -that is, the overshoot became smaller. With 8 to 10 mM $MnCl_2$, the initial rapid rise of the potential was often followed by a dip before it rose to the peak of the plateau (Fig. 2C), and finally the plateau almost disappeared (Fig. 2D). At these concentrations there was also some decrease in the rate of rise of the spike (Table 1), but even the rate of rise in this condition was much larger than that associated with any observable change of the plateau of the spike under tetrodotoxin. The effects of tetrodotoxin and Mn++ were usually reversible.

These findings, if considered in the light of data obtained on fiber of barnacle muscle, are in accord with the idea that the initial rise of the action potential in ventricle fiber of the frog is related to the increase in permeability to Na, while the plateau phase is related to the increase in permeability to Ca. However, this does not exclude the possibility that the changes in permeability to Na are also implicated in the plateau phase.

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Anesthesia of Artemia Larvae: Method for Quantitative Study

Abstract. Potency of anesthetics is quantitatively measured with laboratory-hatched larvae of the brine shrimp Artemia salina. Statistical fluctuations are minimized in that 100,000 animals are used to determine a single median anesthetic dose value. The technique was developed to study molecular mechanisms of general anesthesia.

Accurate values for AD_{50} (the dose necessary to produce anesthesia in 50 percent of animals) are needed to assess the relative merits of the recent molecular theories on general anesthesia of Pauling (1) and Miller (2), and of the theory advanced 65 years ago