values of T_2 in the malignant tumors (0.100 and 0.118 second) were about twice the values of T_2 in rectus muscle (0.055 second) and liver (0.052 second). Furthermore, replicate measurements of T_1 in the malignant tissues were found to be highly reproducible (standard error of the mean, $< .02$) and the normal tissues had a standard error of the mean of .03 or less despite deliberate scrambling of the ages and weights of the animals in the experimental colony.

On the whole, these results support the findings of Hazlewood and his co-workers (*7*) and are in general agreement with Szent-Györgyi's assertion that cancerous tissue has a lower degree of organization and less water structure than normal tissue (*10*). Furthermore, the data conformed to the results expected on the basis of a knowledge of the cation content of cancerous tissue. Dunham *et al.* have reported that with "few exceptions the potassium content of malignant neoplasms is increased" by comparison with that of normal cells (*11*). Ling has pointed out that the variations in alkali cation selectivity observed by Dunham *et al.* are readily explained by the association-induction hypothesis (*5*, p. 523). Nuclear magnetic resonance line width measurements in my laboratory (*6*) have demonstrated a correlation between narrowing of the line width of the cell water signal and potassium enrichment in bacteria (*E. coli*), which, in turn, is consistent with the aqueous properties of potassium as a "structure-breaking" agent (*12*). "Structure-breaking" by the alkali cations below Na in the periodic table (K, Rb, Cs), producing decreased ordering of the molecules in bulk water, results in narrowing of the NMR line width (*6*).

The measurements were also unaffected by any change in the elevation of the sample position in the probe, packing and repacking of the specimen, or the stepwise rotation of the sample tube in the probe through 360°. In fact T_1 proved to be even relatively unchanged after the specimens stood overnight at room temperature (Tables 1 and 2, parenthetical values for rats 4 and 8).

These studies indicate that NMR methods may be used to discriminate between two malignant tumors and a representative series of normal tissues. The results suggest that this technique may prove useful in the detection of malignant tumors.

The possibility that NMR might be used for rapid discrimination between benign and malignant surgical specimens was also considered. Relaxation times for two benign tumors (fibroadenomas) were distinct from those of the malignant tissues and were the same as those of muscle (Tables 1 and 2).

RAYMOND DAMADIAN*
Biophysical Laboratory, Department of Medicine, State University of New York, Brooklyn 11203

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* Career scientist of the Health Research Council of the City of New York.

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Synaptic Transmission in the Crayfish: Increased Release of Transmitter Substance by Bacterial Endotoxin

Abstract. *Bacterial endotoxin increases the frequency of miniature excitatory postsynaptic potentials, decreases facilitation, and increases the evoked excitatory postsynaptic potential without changing membrane resistance. These data indicate that endotoxin acts on the presynaptic nerve terminal by increasing the amount of transmitter substance released in response to an applied stimulus.*

There is considerable evidence that bacterial endotoxins possess neurotoxic properties. When endotoxin is injected into a cerebral ventricle or into the coeliac ganglion, the dose required to evoke fatal hemodynamic collapse is a small fraction of the dose required when it is given intravenously (*1–3*). The pathological effects of endotoxin do not develop in an organ which has been denervated; for example, denervation of one kidney blocks the cortical necrosis of the generalized Schwartzman reaction on that side only (*4*). Similarly, blockade of the coeliac ganglion prevents gastrointestinal injury in the stomach and the entire intestine except for the still innervated distal half of the colon (*5*). Denervation of one-half of the spleen prevents injury to that half only, such that it continues to extract injected endotoxin, whereas the other half does not (*6*). The denervated heart-lung preparation in the dog shows no hemodynamic changes in response to the injection of one or several lethal doses of endotoxin (*7*).

With these data in hand a study of the effect of endotoxin on nerve function was undertaken. We now report evidence that endotoxin acts on the presynaptic nerve terminals causing them to release an increased amount of transmitter substance in response to an applied stimulus.

Observations were made on two neuromuscular preparations of the crayfish (*Orconectes* sp.). The crayfish neuromuscular preparation has been shown to behave like the rat phrenic nerve–diaphragm model in that both demonstrated presynaptic effects in response to a variety of neurotoxins (*8*). The deep abdominal extensor muscles as well as the closer of the claw were prepared in a manner described previously (*9, 10*). The preparation was immersed in a modified van Harreveld solution (*11*) (200 m*M* NaCl, 5 m*M* KCl, 13.5 m*M* $CaCl_2$ *p*H adjusted to 7.4 with tris buffer). After removal of the opener muscle microelectrodes filled with 3*M* KCl were inserted into the closer muscle to record intracellular activity. The corresponding nerve was stimulated at the meropodite by means of fine silver electrodes and a square-wave pulse 0.05 msec long. Similar

preparations were used for recording from the deep abdominal extensor muscles. Membrane resistance was measured directly by two microelectrodes inserted in the same muscle fiber. The current was monitored across a 10-kilohm resistor.

Endotoxin of *Serratia marcescens* [the lethal dose for 50 percent of mice tested (LD_{50}) is 610 μg (*12*)] dissolved in crayfish saline was added to the bath to produce a final concentration of 1 to 2 μg per milliliter of bath solution. The final concentration of endotoxin in the bath approximates that of the recent measurements of plasma endotoxin after injection of one LD_{50} of endotoxin in the rabbit (*13*).

Figure 1 shows the intracellular responses of the muscle to nerve stimulation. The control postsynaptic potential was 3.3 mv (Fig. 1A), and its time constant in the decaying phase was 14.5 msec. One minute after the application of endotoxin the postsynaptic potential increased to 7.3 mv (Fig. 1B), but the time constant was slightly reduced (12.0 msec). During the next 4 minutes the postsynaptic potential rose gradually to 8.7 mv (Fig. 1D), at which time an electrically excitable response appeared (Fig. 1, D–F). On successive washing with crayfish saline the postsynaptic potential returned to near its control value (Fig. 1, G–I).

This result might be accounted for by an increase in the resistance of the muscle membrane, an increase in the efficacy of the transmitter, or an increase in the amount of transmitter released per impuse. Since the time constant showed a slight decrease in spite of a large increase in the maximum amplitude of the postsynaptic potential, it appears that there is no change in membrane resistance. Direct measurements of membrane resistance in response to hyperpolarizing current before and after the application of endotoxin supports this conclusion. Figure 2 shows that the membrane resistance did not change upon exposure of the preparation to endotoxin for up to 20 minutes.

Miniature postsynaptic potentials were recorded to determine if endotoxin increases the efficacy of the transmitter substance or the amount released. The frequency of the miniatures increased three- to fourfold from 0.21 to 0.73 per second (Table 1). The miniature junction potential remained unchanged (13 to 20 μv) in 74 percent and increased to a maximum of 25 to 32 μv in 26 percent after addition of endotoxin. Although changes in the amount of transmitter released and its efficacy cannot be completely ruled out, the doubling of the magnitude in 26 percent of the miniature junction potentials after addition of endotoxin, coupled with the increased frequency, and the lack of change in membrane resistance indicate that the increase in amplitude represents doublet miniatures. This evidence therefore favors a presynaptic effect of endotoxin.

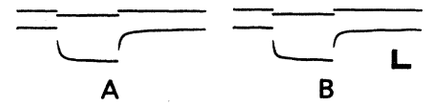

Fig. 2. Measurements of membrane resistance in abdominal extensor muscles before (A) and after (B) addition of endotoxin (2 μg/ml). Upper beam, current; lower beam, membrane potential. Calibration scale: 20 msec, 50 mv, 2 μa.

Table 1. Miniature junction potential (mjp) in the crayfish claw closer. Miniature potentials in the control and after addition of endotoxin were recorded for periods of 75 consecutive seconds from which the amplitude and frequency were calculated.

Condition	Microvolts					Frequency (mjp/sec)
	13–16	17–20	21–24	25–28	29–32	
Control	8	8	0	0	0	0.21
Endotoxin	32	9	0	10	4	0.73

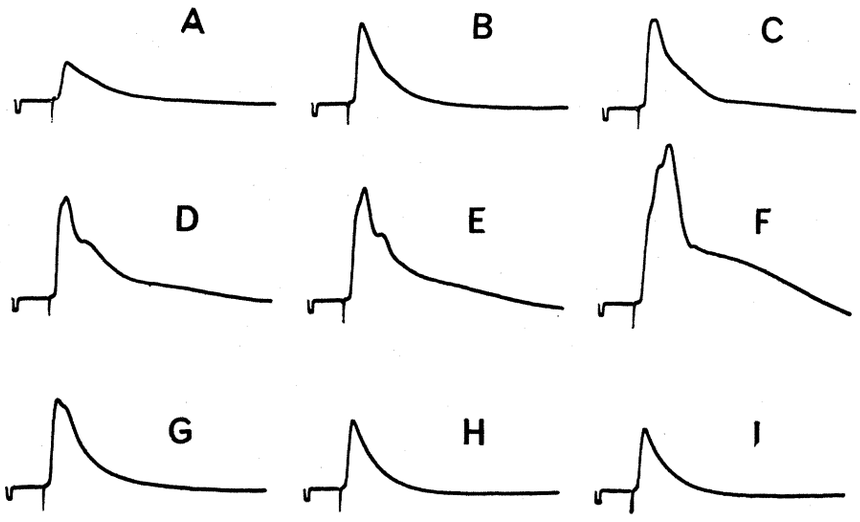

Fig. 1. Effect of endotoxin (2 μg/ml) on the postsynaptic potential in the abdominal extensor muscles of the crayfish. (A) Control; (B) 1 minute after addition of endotoxin; (C) 3 minutes after addition of endotoxin; (D) 5 minutes after addition of endotoxin; (E) 7 minutes after addition of endotoxin; (F) 9 minutes after addition of endotoxin; (G) partial recovery after first washing; (H) partial recovery after second washing; (I) partial recovery after third washing. Calibration pulse 2 mv, 2 msec. Note electrogenic response in D through F.

Rahammimmoff (*14*) showed that the degree of facilitation in twin pulse experiments depends on the amount of transmitter released in the first response. One might therefore anticipate that endotoxin would alter facilitation. The control values of the postsynaptic potentials for the first and second responses in such twin pulse experiments were 3.10 ± 0.11 mv and 5.12 ± 0.32 mv, respectively, and the facilitation ratio (V_2/V_1) was 1.64. During the first 5 minutes after the application of endotoxin both V_1 and V_2 increased to 5.52 ± 0.14 mv and 7.20 ± 0.17 mv, respectively, whereas the facilitation ratio decreased to 1.30. Further incubation with endotoxin resulted in increases in V_1 and V_2 to 6.06 ± 0.08 mv and 7.85 ± 0.13 mv, respectively, but the facilitation ratio remained stable at 1.30. This statistically significant ($P < .01$) reduction in the degree of facilitation is ascribable to an increase in the amount of transmitter released at the first response, and is consistent with the conclusion that the site of action of endotoxin is presynaptic.

The data presented here with respect to changes in postsynaptic potential and facilitation, in the absence of changes in membrane resistance, lead to the

conclusion that endotoxin acts on the nerve terminal by increasing the number of quanta released in response to an applied stimulus.

I. PARNAS*
R. REINHOLD
J. FINE

Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York, and *Harvard Surgical Unit, Boston City Hospital, Boston, Massachusetts 02118*

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* Present address: Department of Zoology, Hebrew University, Jerusalem, Israel.

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A Correlation between Gametophytic and Sporophytic Characteristics in Zea mays L.

Abstract. *If a mixture of types of corn pollen, identified by genetic markers, is applied to the silks of other inbred lines, the rate of pollen tube growth often varies with type of pollen. This gametophytic differential is correlated with a sporophytic differential—relatively heavier seeds in seed mixtures result from fertilization by gametes from faster growing tubes. The increased seed weight is due to greater competitive ability of the zygotes thus formed.*

Differential rates of pollen tube growth have been described in several plant species (*1*). The selective consequences of such differentials could be great, but only if a substantial proportion of the genetic system functions in both gametophytic and sporophytic phases of the life cycle (*2*). This study demonstrates, in *Zea mays* L., a significant relationship between a gametophytic character, relative speed of pollen tube growth, and a sporophytic character, relative seed weight. Thus, it must be concluded that some genetic factors expressed in the gametophyte function also in the sporophyte.

The analysis is based on the observation (*3*) that if a mixture of pollen types is applied to stigmas and if these types, tagged by markers, penetrate stylar tissues at different relative speeds, then the probability of the faster type reaching ovules first will be proportional to the style length. This is because not all pollen tubes start at the same position. Accordingly, random effects will be more significant when styles are short. With long styles, relative speed of growth will outweigh these random effects. Since the styles for basal kernels on an ear of corn are longer than the styles of apical kernels, any basipetal increase in frequency of fertilization by one pollen type will be a measure of that pollen type's greater relative speed. If the composition of the resultant seed mixture is determined at different points within the ear, the slope of a regression of percentage of one seed type against distance from the apex will express the relative speed of the pollen tubes associated with that seed type. This, of course, assumes that both types are capable of reaching the basal ovules.

Two marker systems, each represented in several inbred lines, were used; one for white (*yy*) or yellow (*Y-*) endosperm and another for aleurone color (A_1-) (or a_1a_1 for colorless). Pollen from one dominantly marked plant and one recessively marked plant were mixed in approximately equal proportions and then applied to silks of recessive lines (other than that in the pollen mixture). Components of the mixtures were also applied singly to the same recessive lines. Mixed crosses were replicated five times, unmixed crosses were replicated three to five times; unfilled ears were discarded. The ears were divided transversely into five nearly equal segments; seeds from the mixed crosses were separated according to color, and average weights were obtained. In all, 30 ears in eight mixtures were analyzed. Ears pollinated by a single pollen type were treated similarly.

The regression for change in percentage composition of each seed mixture from apex to base was calculated. The slopes of the positive regression in each mixture (the increasing, thus faster type) were taken as a measure of the relative speed of pollen tube growth.

Relative seed weight was the sporophytic character selected for study. Average seed weight is an indicator of heterosis (*4*), at least when compo-

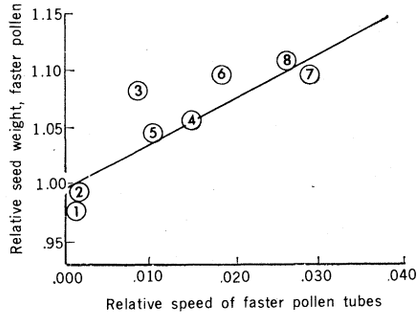

Fig. 1. Relation between relative speed of pollen tubes and the relative average weight of resultant seeds. Relative speed of the faster pollen is expressed as slope of regression for change in proportion of seed type from apex to base of the ears. Relative average seed weight with faster pollen as staminate parent is the average seed weight from the faster divided by average seed weight from the slower. The regression is significant at the 2 percent level, with a *Y*-intercept of 0.9951 ($t = 3.672$, d.f. $= 6$, $R = 0.8319$). Mixes 5, 6, and 8 (circled numbers) included five replications each, mix 2 included four, mixes 1, 3, and 4 included three replications each, and mix 7 included two. If all 30 replicates are plotted separately, a regression also significant at the 2 percent level is obtained (*Y*-intercept, 1.017; $t = 2.520$, d.f. $= 28$, $R = 0.4299$). The lower R value likely reflects not only the smaller size of each sample, but also the finding (*7*) that within highly inbred populations there may be significant heterogeneity between families.