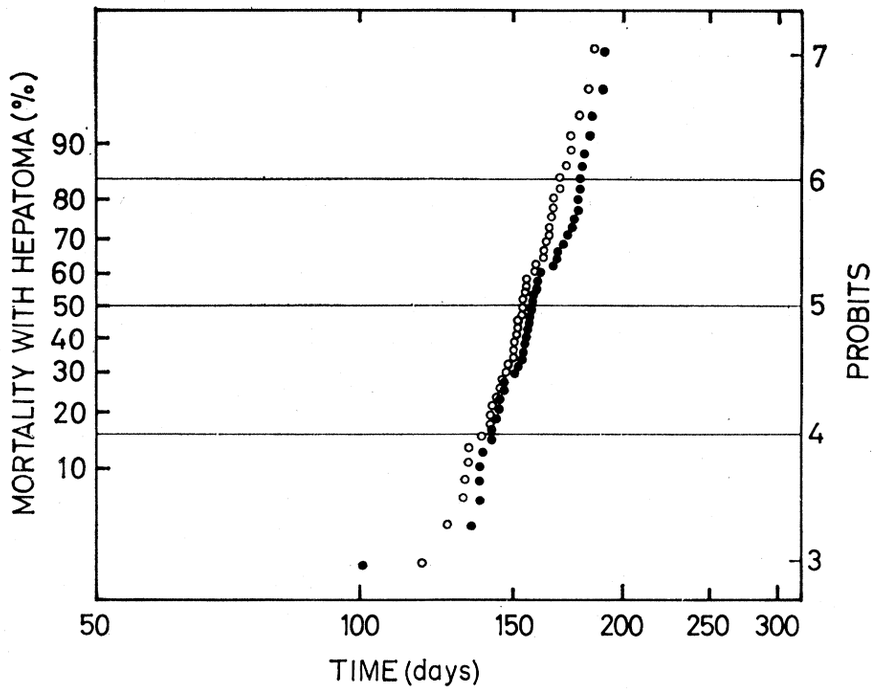

Fig. 4. Effect of daily low-dose x-irradiation on tumor development in the DEN–rat liver system. Mortality with liver carcinomata is plotted against time in a probability grid [experimental points corrected after the method of Miescher *et al.* (*14*)]. Treatment: (●) 5 mg of DEN per kilogram per day; and (○) 5 mg of DEN per kilogram per day plus 5 r per day. Each point represents one animal.

rapid proliferation restore the liver mass to its original size within a short time after operation (*10*).

The effect of daily low doses of irradiation on response of the liver to DEN carcinogenesis was studied in another series of experiments. In Wistar rats treated with daily doses of 3 mg of DEN per kilogram of body weight and irradiated with a single dose of 320 roentgens before starting the experiment, there was no statistically significant effect on T, although T for irradiated animals was slightly shorter than for animals treated with DEN only (*11*). We administered daily doses of 5 mg of DEN per kilogram combined with daily doses of 5 r (±3 percent S.D.) (*12*) whole-body irradiation.

Four groups of 50 adult, male Sprague-Dawley rats each (mean body weight, 290 g ± 10 percent S.D.) were used: group O was the untreated control, group A received irradiation only, group B was given DEN only, and group C was treated with DEN plus irradiation. According to the generally accepted observation that body weight of rats is a very sensitive indicator of radiation (*13*), reduction of mean body weight by additional low-dose irradiation is reflected in Fig. 3. The reduction factor is very nearly the same for the untreated control group O and for group B which was treated with DEN only. Hence, concerning reduction of body weight, there is summation of the effects of DEN and x-rays. Figure 4, however, shows that there is no significant difference in T between the combined group C and group B, although, as in an earlier experiment (*11*), there is a slight shift to the left for group C. There may be a better chance to demonstrate radiation effects on this system of experimental carcinogenesis by lowering the daily dose of DEN in order to increase T in the unirradiated group.

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1 February 1966

Lithium's Failure to Replace Sodium in Mammalian Sympathetic Ganglia

Abstract. *Ganglionic responses to electrical stimulation, acetylcholine, and potassium ions were studied in superior cervical ganglia of cats perfused with media containing lithium chloride instead of sodium chloride. In lithium-Locke, ganglionic transmission and depolarization evoked by acetylcholine were blocked completely but reversibly, while the depolarization produced by potassium ions was unaltered.*

There is much evidence that acetylcholine depolarizes neuronal and junctional tissues primarily by causing a marked increase in sodium permeability at those sites and that it also causes an increase in permeability of these tissues to other cations (*1*). In peripheral nerve and sympathetic ganglia, complete replacement of sodium ions in the bathing medium with iso-osmotic equivalents of calcium ions maintains acetylcholine-induced depolarization until such time as stabilizing action of the divalent cation exerts itself. Moreover, the tissues retain considerable sensitivity to acetylcholine when sodium in the medium is replaced by nonelectrolytes. Under these conditions, removal of calcium from the bathing medium results in loss of sensitivity to acetylcholine. When acetylcholine is applied to these tissues in the absence of sodium ions, it is apparently the movement of calcium ions into the cells that provides current necessary for discharge

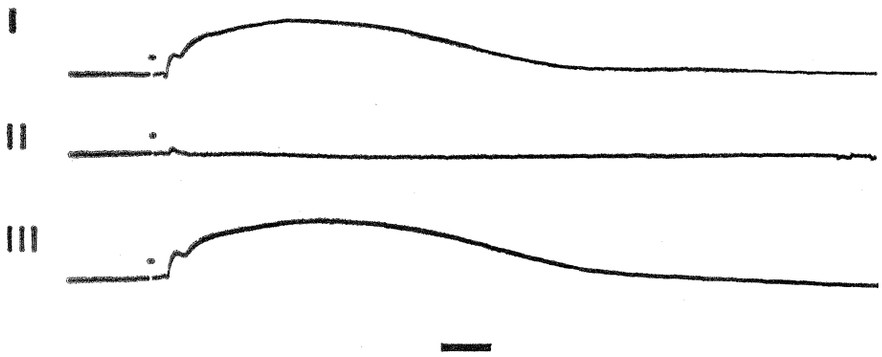

Fig. 1. Comparison of ganglionic responses to injected acetylcholine (20 μg) in sodium-Locke and lithium-Locke solutions. (*I*) Ganglionic depolarization produced by 20 μg of acetylcholine in standard Locke solution. (*II*) Blockade of acetylcholine-induced ganglionic depolarization 31 minutes after 154 m*M* LiCl replaced 154 m*M* NaCl. (*III*) Recovery of ganglionic depolarization evoked by acetylcholine 25 minutes after reintroduction of 154 m*M* NaCl. The vertical calibration on each record is 1 mv and signals the injection of acetylcholine. The horizontal calibration is 4 seconds.

of the membrane potential. Similar observations have been made at the motor end-plate of skeletal muscle (*1*).

The ability of lithium to fulfill the role of sodium in conducting membranes has been explained in a similar way. Lithium ions can completely substitute for sodium ions in mechanisms underlying the generation of action potentials in squid giant axon, frog skeletal muscle fibers, and unmyelinated mammalian nerve fibers (*2*). However, the question of whether lithium can effectively replace sodium at junctional tissues that are sensitive to depolarization by acetylcholine has not received much attention. Hence, we have compared the effects of replacing sodium ions with lithium ions on responses of perfused sympathetic ganglia of cats to electrical stimulation of the preganglionic nerve, to injected acetylcholine, and to injected potassium chloride.

Superior cervical ganglia of 15 cats, anesthetized with a mixture of sodium diallylbarbiturate and urethane, were prepared for perfusion with media of varying electrolyte composition and for electrophysiological recording in a manner that we described earlier (*1*). The cervical sympathetic nerve and the external carotid postganglionic nerve were dissected free from underlying connective tissues. Bipolar platinum-wire electrodes were used for stimulation of the cervical sympathetic trunk. Supramaximal shocks of 0.1-msec duration were applied every 2 seconds. Ganglionic action potentials and drug-evoked responses were recorded from the surface of the ganglion by means of bipolar silver–silver chloride electrodes. One electrode was placed in contact with the body of the ganglion, the other on the crushed end of the postganglionic nerve. Permanent records were obtained by photographing responses as they were displayed by an oscilloscope. In order to limit perfusion fluid to the ganglion, all branches of the common carotid artery, except those supplying the ganglion directly, were tied. Perfusion solutions were introduced into the vasculature of the ganglion by means of a cannula inserted into the common carotid artery. Perfusion of the ganglion was accomplished with a constant-delivery infusion pump that was set to operate at a rate of 0.4 ml/min. Drug injections were made through a 27-gauge needle located immediately anterior to the perfusion needle. Standard sodium-Locke solution used for perfusion was a bicarbonate-buffered saline medium of the following composition: 154 m*M* sodium chloride, 6 m*M* sodium bicarbonate, 5.6 m*M* potassium chloride, 2.2 m*M* calcium chloride, and 5.5 m*M* glucose. In the sodium-deficient solutions (lithium-Locke), 154 m*M* lithium chloride was used. All of the solutions were gassed with a mixture of 96.5 percent oxygen and 3.5 percent carbon dioxide and passed, under pressure, through a fritted glass filter of medium porosity. The *p*H's of the solutions ranged from 6.8 to 7.0.

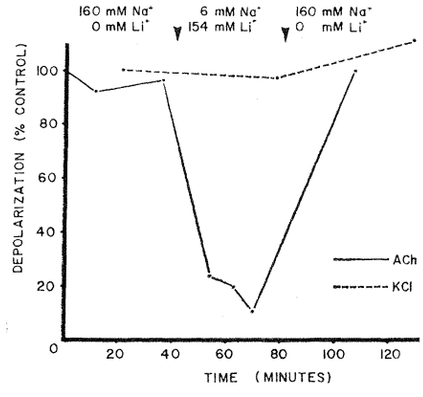

Fig. 2. Graphic illustration of the time-course of ganglionic responses to acetylcholine (20 μg) and potassium chloride (1 mg) in sodium-Locke and lithium-Locke solutions. Ordinate indicates area of drug-induced depolarization relative to control response. Between the arrows perfusion with lithium-Locke solution caused profound depression of acetylcholine-induced ganglionic depolarization while that evoked by potassium chloride was unchanged. Rate of recovery of ganglionic response to injected acetylcholine in standard Locke solution is comparable to rate of onset of blockade in lithium-Locke solution.

As we have reported elsewhere (*1*), ganglionic transmission and the responses to injected acetylcholine were supported for 2 to 3 hours in ganglia perfused with standard sodium-Locke solution. Complete replacement of sodium chloride with an iso-osmotic equivalent of either glucose or sucrose resulted in a complete failure of transmission 6 minutes after onset of perfusion with this medium. Similarly, ganglionic depolarization evoked by injected acetylcholine (20 to 40 μg, into the perfusion stream) was severely depressed during the first 7 to 12 minutes of perfusion with sodium-deficient medium. However, after this period of time acetylcholine was able to evoke significant depolarization of the ganglion. That acetylcholine-induced depolarization which occurred in ganglia deprived of extracellular sodium ions was due to calcium ions is suggested by the finding that response to acetylcholine was abolished when calcium ions were also removed from the perfusion fluid.

When the sodium chloride of the perfusion solution was replaced with lithium chloride (154 m*M*) rather than with nonelectrolytes, a different pattern of responses to injected acetylcholine occurred. In each of ten experiments with ganglia perfused with lithium-Locke solution, ganglionic transmission and response to injected acetylcholine (20 to 40 μg) were depressed 8 to 10 minutes after the onset of perfusion and completely blocked within 35 minutes (Fig. 1). In contrast to results obtained with ganglia perfused with solutions containing glucose or sucrose as a replacement for sodium chloride, gan-

glionic depolarization induced by acetylcholine did not reappear when perfusion with lithium-Locke solution was extended beyond 10 minutes (Fig. 2). However, in each of eight experiments depolarization of the ganglia produced by potassium chloride (1 mg) was not materially altered during perfusion with lithium-Locke solution (Fig. 2). Both ganglionic transmission and acetylcholine-induced depolarization returned to control value upon reintroduction of sodium-Locke solution. The time-course of the several changes produced by lithium ions in ganglionic activity is illustrated graphically in Fig. 2.

In view of work by others (*2*), it can be assumed that conduction of impulses in the pre- and postganglionic neurons of the ganglia would be essentially normal during the period of perfusion with lithium-Locke solution. Inability of lithium to support transmission of impulses in sympathetic ganglia can be attributed, therefore, either to a decrease in release of acetylcholine from nerve terminals or to failure of lithium to substitute for sodium at the postjunctional membrane. Although it is not possible at present to decide between the alternatives, it is noteworthy that failure of transmission and the depolarization of ganglion cells by injected acetylcholine had a parallel time-course.

One of the most important differences in the cellular disposition of sodium and lithium is the relative difficulty with which the latter is extruded from intracellular sites (*2*). Therefore, it is possible that the failure of lithium to support ganglionic responses to acetylcholine is due to persistent ganglionic depolarization that results from accumulation of lithium ions within the ganglion cells. It has been shown that a low-amplitude, sustained depolarization of the superior cervical ganglion produced by tetanic preganglionic stimulation, pilocarpine, or anticholinesterase agents will suppress the depolarization evoked by injected acetylcholine (*3*). Because of the inherent property of the resistance coupled preamplifier to drift several hundred microvolts in 30 minutes, it was not possible to determine directly whether the ganglion was depolarized by lithium-Locke solution. It can be reasonably argued, however, that if the ganglion cells were depolarized as a result of an intracellular sequestering of lithium ions, then the ganglionic depolarization produced by potassium chloride, like that evoked by acetylcholine, should have been depressed. Since there was no change in ganglionic response to potassium, it is unlikely that ganglion cells were depolarized by lithium.

By exclusion, it appears that the inability of lithium to substitute for sodium in the superior cervical ganglion was due to the fact that mechanisms involved in the acetylcholine-induced ganglionic depolarization are able to discriminate between the two ions. This is in marked contrast to those mechanisms concerned with conduction of impulses in peripheral nerve and in skeletal muscle fibers, although the difference between junctional and conducting tissues with regard to the ability of lithium to substitute for sodium may be only quantitative in nature.

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27 December 1965

Ornithine Transcarbamylase Enzymes: Occurrence in Bacillus licheniformis

Abstract. *Two separate ornithine transcarbamylase enzymes have been found in extracts of late exponential-phase cells of* Bacillus licheniformis *grown on glucose and* L-*arginine. One enzyme presumably has a biosynthetic function and is repressed by arginine. The other is induced by arginine, is relatively heat-stable, and can be separated from the first by diethylaminoethyl chromatography.*

A recent report (*1*) demonstrated that ornithine transcarbamylase (OTC) (*2*) activity could be both repressed and induced by arginine in late exponential-phase cells of *Bacillus licheniformis*. Since it was clearly established that the induction of the enzyme activity could not be a derepression, a control system that would allow both events to occur in a single cell was difficult to envisage. Such a phenomenon has never been demonstrated; therefore it was important to determine whether the repressible and inducible OTC activities found in extracts of *B. licheniformis* have separate or identical physical identities.

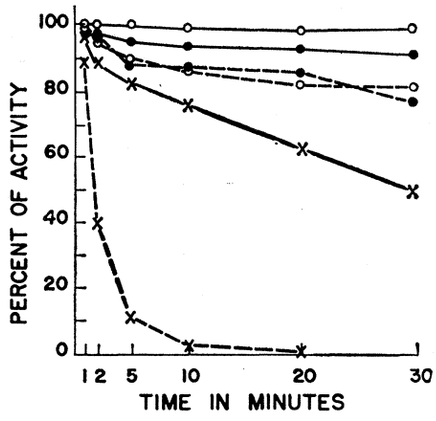

Fig. 1. Heat denaturation of induced and normal enzyme activities. Enzyme extracts were diluted to about 0.4 unit of OTC activity per milliliter in 5 m*M* tris-HCl buffer, *p*H 7.5, containing 1.0 m*M* mercaptoethanol (TM buffer) and incubated at 22°, 37°, and 55°C. At the times indicated, 0.1-ml samples were removed and immediately diluted to 0.9 ml with a solution (at 0°C) containing 100 μmole of tris-HCl buffer, *p*H 8.5, and 5 μmole of L-ornithine. After warming the solution to 37°C and adding 5 μmole of carbamyl phosphate, the OTC activity was then determined. Open circles, 22°C; closed circles, 37°C; *x*'s, 55°C; broken lines, induced activity; solid lines, normal activity.

Bacillus licheniformis, strain A-5, was grown in 1-liter lots to late-exponential phase in a minimal medium (*3*) with 20 m*M* glucose and 50 m*M* ammonium lactate as the carbon and nitrogen sources. Cells were harvested and washed twice at 0°C by centrifugation at 10,000*g* in a Sorvall RC-2 centrifuge. They were suspended in 2 m*M* tris-HCl buffer, *p*H 7.5, containing 1 m*M* mercaptoethanol and were disrupted for 2 minutes in a 20-kc MSE sonic oscillator. The crude extract was centrifuged at 0°C for 45 minutes at 37,000*g* and the supernatant solution was dialyzed against several changes of the same buffer and then frozen. The OTC activity in these extracts ranged from 0.2 to 0.5 unit per milligram of protein, one unit being defined as that amount of activity producing 1.0 μmole of citrulline per minute when measured by the method of Archibald (*4*). Protein concentration was determined by the