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14. For each amine, the amount of reagents added at the end of reaction, the volume of CH_2Cl_2 or ether, the wavelength (and the molar absorptivity) where absorbance was measured, the extraction efficiency, and the nitrosamine formed were as follows: morpholine, 2 ml of 5*N* NaOH, 2 × 10 ml CH_2Cl_2, 358 nm (106), 94 percent, nitrosomorpholine; piperazine, 4 ml of 5 NaOH and 2 g of NaCl, 4 × 15 ml of CH_2Cl_2, 358 nm (114), 86 percent, mononitrosopiperazine [kinetic studies (*5*) show that mono- and not dinitrosopiperazine is the main initial product]; *N*-methylaniline, 15 ml of saturated sodium tetraborate solution adjusted to *p*H 9, 3 × 10 ml of CH_2Cl_2, 369 nm (227), 100 percent, *N*-methyl-*N*-nitrosoaniline; dimethylamine (experiments 15 to 18), 5 ml of 5*N* NaOH and 2.5 g of NaCl, 4 × 35 ml of ether, 358 nm (119), 80 percent, dimethylnitrosamine; dimethylamine (experiments 19 to 21), temperature (37°C) and other reaction conditions as for oxytetracycline experiments, workup as for experiments 15 to 18.
15. We thank J. Sams for technical assistance, E. Conrad for running the gas chromatograms, and Pfizer Corporation, New York, for oxytetracycline. Supported by contract PH 43-68-959 from the National Cancer Institute and by grant BC-39 from the American Cancer Society.

14 February 1972; revised 18 April 1972

Decreased RNA Polymerase Activity in Mammalian Zinc Deficiency

Abstract. *The activity of DNA-dependent RNA polymerase has been measured in liver nuclei from suckling rats nursed by zinc-deficient dams, or by controls that were either pair-fed or given free access to the diet. In the zinc-deficient pups, the activity of the enzyme did not increase; it fell after the tenth day of life.*

Zinc is an essential nutrient that appears necessary for the synthesis of nucleic acids (*1–3*) and protein (*4*). The manner in which it influences these processes is unknown. Its requirement for the action of various metalloenzymes is well known (*5*), and its influence on the activity of mammalian (*1*) and *Escherichia coli* (*6*) DNA polymerase has been reported. While zinc may play a role in the maintenance of the conformation of nucleic acids (*7*), and zinc nutriture of the animal influences the sucrose density gradient profile of liver polysomes as well as the incorporation of uridine into those polysomes (*8*), it is far from clear how these latter events take place. Therefore, the influence of zinc deficiency on the activity of DNA-dependent RNA polymerase in nuclei of liver cells has been assessed in suckling rats.

Feeding a biotin-enriched diet containing sprayed egg white (20 percent by weight) (*3*) to pregnant dams from the 18th day of gestation through the 16th day of the neonatal period produced zinc deficiency in their pups. Pair feeding of the zinc-supplemented dams (160 μg every other day, by intraperitoneal injection) also resulted in growth failure in their pups. The zinc supplementation was sufficient to maintain normal growth in pups nursed by dams given free access to an adequate diet (Fig. 1). Similar observations have been reported by Mutch and Hurley (*9*), who also found that the concentration of zinc in milk from zinc-deficient dams is decreased along with a decrease in total volume. In contrast, they found that milk from pair-fed dams has increased concentrations of zinc and protein while the volume is decreased. Growth retardation in pups nursed by pair-fed dams therefore reflects the fewer calories available to the pups as a consequence of maternal starvation.

The litters of the dams were sampled at intervals, the sampled pups were decapitated, and their livers were excised and pooled (3 to 11 pups per assay, with the usual number being 5 or 6) The effect of zinc deficiency, starvation, and free access to food on the activity of nuclear DNA-dependent RNA polymerase was measured. The method of Widnell and Tata (*10*) was used to prepare the nuclear suspensions. Enzyme activity was assayed as described by Weiss (*11*). The [^{14}C]adenosine triphosphate incorporated into RNA formed on the nuclear DNA was assayed by liquid scintillation counting and was reported in relation to the amount of deoxyribose (*12*) present. By duplicate assays, these techniques were found to have a high degree of reproducibility; in addition, the activity of the enzyme from pups of the same age and treatment was also similar.

Our studies indicate that starvation (pair feeding) did not inhibit the activity of the enzyme while zinc deprivation did (Table 1). In fact, the activity of the enzyme showed a steady decline in the pups deficient in zinc from day 10 of life ($P < .001$).

These observations are the first, of which we are aware, to show the requirement of zinc for the activity of nuclear DNA-dependent RNA polym-

Table 1. Effect of zinc deficiency on activity of liver nuclear DNA-dependent RNA polymerase of suckling rats. The activity of the enzyme rose very little from birth to day 10 of life and then decreased. Pair feeding did not suppress the activity of the enzyme.

Age (days)	Carbon-14 per milligram of deoxyribose (count min^{-1} mg^{-1} ml^{-1})		
	Zinc-deficient	Controls	
		Pair-fed	Free access to food
2	83.0*	81.5	78.6
4	85.9*		90.9
5		91.5	
6	85.0	87.0	94.8
7			103.5
8		98.9	105.0*
10	91.2	111.3*	
11			115.9*
12	80.4	114.4*	112.8
14	70.0	112.1	115.4†
15	64.8	111.1*	112.8*
16	60.4	107.8	107.4

* Average of two sets of pups. † Average of three sets of pups.

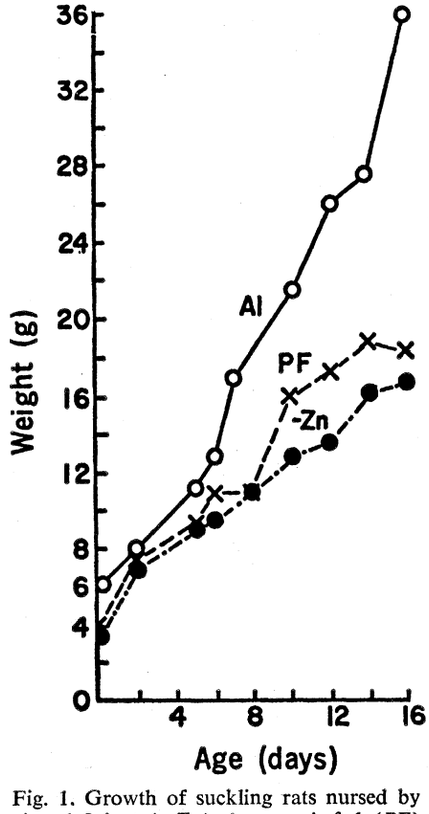

Fig. 1. Growth of suckling rats nursed by zinc deficient (*—Zn*) dams, pair-fed (*PF*) dams, and dams given free access to a normal diet (*Al*). Both zinc-deficient and pair-fed pups showed poor weight gain.

erase in mammalian liver. Of interest in this regard is the recent report by Scrutton *et al.* (*13*) that RNA polymerase of *E. coli* is a zinc metalloenzyme. Our findings complement this report. Taken together, these two reports may help solve part of the puzzle of the role of zinc in the metabolism of RNA.

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14. Supported by a Future Leaders grant from the Nutrition Foundation to H.H.S. and an NSF fellowship to M.W.T.
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4 February 1972; revised 4 April 1972

Antidromic Action Potentials Fail to Demonstrate Known Interactions between Neurons

Abstract. *An identified motor neuron in the stomatogastric ganglion of* Panulirus interruptus *inhibits four other motor neurons when it fires spontaneously or in response to depolarization of its soma. It does not inhibit these neurons when it is fired antidromically, although the attenuated antidromic spike is visible at its soma. These findings point out the difficulty of interpreting negative results from antidromic stimulation experiments and the importance of neuronal structure to the integrative activities of nervous systems.*

Antidromic action potentials are action potentials that start at some unusual site on an axon and travel back toward the normal site of initiation. In the laboratory, such potentials commonly arise because an experimenter stimulates the axon electrically, trying to get such a potential to invade the integrative regions of the neuron. One reason for doing so is to look for interactions between the neuron stimulated and other neurons being monitored during the stimulation [for example, see (*1*)]. The assumption one would like to make is that antidromic potentials will behave in the neuron as do orthodromic potentials, those which arise at the usual initiation site and travel in the usual direction down the axon. According to this assumption, if antidromic potentials reveal interactions between neurons, these interactions also occur when orthodromic spikes arise, and if antidromic potentials do not reveal interactions between neurons, these interactions do not occur when orthodromic spikes arise. This report shows that, in one case, the assumption does not hold, and so challenges its validity in general.

The stomatogastric ganglion of the spiny lobster, *Panulirus interruptus*, has 30 to 36 neurons; it lies on the dorsal side of the stomach in the lumen of the dorsal aorta and controls the numerous striated muscles that move different sections of the stomach. These neurons are monopolar; each neuronal soma is connected to the integrative regions of the neuron by a neurite that does not support a conducted action potential. The spike-initiating zone, the axon, and the branches that form synapses with other neurons are all removed from the soma. The ganglion is an ideal system for testing the logic of antidromic stimulation, since we can regularly record subthreshold synaptic activity as well as attenuated action potentials from the somata of identified neurons, and simultaneously record the action potentials of these neurons in the peripheral nerves. Careful dissection produces a preparation in which the axons of many neurons are separately accessible and can be used for both recording and antidromic stimulation. Using this system, we have shown that inhibitory postsynaptic potentials (IPSP's), which always occur in four identified cells when a particular fifth cell fires spontaneously or in response to depolarization of its soma, do not occur when the fifth cell is invaded by an antidromic action potential originating outside the ganglion.

The stomatogastric ganglion and its associated nerves were dissected free of the stomach. Each nerve was identified by its route from the ganglion to the muscles that it innervated, by use of the anatomic description by Dando and Maynard (*2*). The isolated ganglion and nerves were then pinned out in a Sylgard-lined petri dish. The ganglion was desheathed and transilluminated. The preparation was maintained during dissection and subsequent experimentation in a saline solution containing 521 m*M* Na^+, 10 m*M* K^+, 16.7 m*M* Mg^{2+}, 16.7 m*M* Ca^{2+}, 21 m*M* SO_4^{2-}, 557 m*M* Cl^-, and 3 m*M* *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid. The *p*H was adjusted to 7.3 to 7.5 with NaOH. The saline was aerated overnight before use, and 2 m*M* glucose was added at the start of the experiment. During the dissection, the saline was cooled to about 10°C. The experiments were performed at room temperature, 20°C.

Action potentials were recorded from the nerves with 100-μm stainless steel electrodes pinned into the Sylgard in a monopolar configuration (*3*). The potentials were differentially amplified, displayed on an oscilloscope, and recorded on a frequency-modulated tape recorder for subsequent filming. The recording electrodes could also be used for antidromic stimulation; a simple switching network allowed us to change the function of the electrode without disturbing the preparation. The stimuli were isolated from ground.

Intracellular recordings were made by desheathing the ganglion and then penetrating the somata of particular cells with glass capillary microelectrodes filled with 2.5*M* KCl. Electrode resistances varied from 20 to 50 megohms. The intracellular potentials were observed through a W-P Instruments M4A preamplifier, displayed on an oscilloscope, and recorded on a frequency-modulated tape recorder. The membrane potentials of the cell could be controlled by passing current through the microelectrode by means of the current-injection feature of the preamplifier.

The isolated stomatogastric ganglion