Zinc deficiency and lung converting enzyme activity in rats

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Summary. Angiotensin converting enzyme activity was found to be significantly decreased in the isolated perfused lung from zinc-deficient rats when compared with that of controls. Addition of zinc ion to the superfusion medium did not cause a recovery in this decreased activity of the enzyme. It is postulated that zinc deficiency probably produces a structural change in the lung angiotensin converting enzyme.

High levels of angiotensin converting enzyme (ACE) activity has been found in the pulmonary[2] and mesenteric[3] circulation. This enzyme(s) is a specific component of vascular endothelium in most mammalian organs[4], especially plasma membrane of endothelial cells in the lung[5]. ACE could be separated from kininase II, responsible for the removal of 'Phe-Arg' from C-terminal end of bradykinin[6]. Recently a new ACE has been found in the hog and guinea-pig plasma without kininase activity[7]. The results of in vitro studies indicate that this new enzyme can be activated by Co^{2+}, Ca^{2+} and Zn^{2+} ions[7,8]. No information, however, is as yet available concerning the conversion of angiotensin I (A I) to angiotensin II (A II) in the pulmonary vascular bed from zinc-deficient animals. The results of the present study indicate that the conversion of A I to A II in the pulmonary circulation of the isolated perfused lung from zinc-deficient rats is significantly decreased when compared with the conversion occurred in the lung from normal animals.

Material and method. The experiments were performed on 16 female rats. 6 rats were fed with normal diet and given ordinary drinking water. 10 rats were separately maintained in stainless steel cages and given deionized water in glass bottles. These animals were fed with a special diet containing 2.2–3 ppm Zn^{2+}[9]. 4 weeks after starting the zinc-deficient diet, the animals were anesthetized with ether and their lungs were isolated perfused as described previously[10]. The venous outflow was continuously superfused over the rat ascending colon[11]. The isotonic contractions of the colon were recorded on a smoked drum by means of frontal levers with 12-fold magnification. In each experiment, the dose-response relation of A II and A I were first determined on the colon, then A I was given through the pulmonary artery, the venous outflow superfused over the assay organs and the responses were recorded. Conversion of A I to A II was calculated, comparing the response of the colon to the known molar concentrations of the peptides. The zinc level of the bone was taken as a criterium and measured in femur by an atomic absorption spectrophotometer (Perkin-Elmer, model 103) after appropriate methods of preparations[12]. The b.wt was determined weekly in both groups. Decapeptide A I (human angiotensin I, 2 acetate, 4 H_2O; Bachem Inc. Chemicals, USA), octapeptide A II (Asp[1]-β-amide-Val[5]-Phe[8]-angiotensin II, Hypertensin, Ciba-Geigy, Switzerland) were dissolved in Krebs' solution from their stock solutions prepared in 0.1 N acetic acid (100 µg/ml). The results were statistically evaluated using Student's t-test.

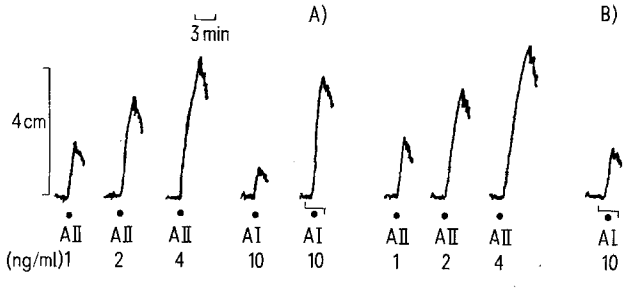
Results. The typical symptoms of zinc deficiency occurred within the 2nd week following the application of zinc-deficient diet to the animals and reached to maximum at 4th week. These symptoms were usually characterized by hair loss, growth retardation, fissure at the corners of the mouth, allopesia and dermal lesions. The b.wt decreased significantly when compared with that of controls. The zinc content of the bone was also found to be decreased (table). The figure shows a typical recorder tracing indicating the response of the rat colon to A I given through the pulmonary artery of the isolated perfused lung from normal rat (A) and zinc deficiency rat (B). The percent conversion of A I to A II during single passage through the lung was calculated as 26.8 in the isolated perfused lung from normal rats. This value, however, was found to be 4.7 in the lung from zinc-deficient rats (table). No recovery was obtained in the ACE activity after adding Zn^{2+} to the superfusion medium at the concentration equal to that of normal serum level (109.3±6.8 µg/100 ml, n=50).

Discussion. The data obtained from this dynamic assay study indicate that zinc deficiency causes a significant decrease in the activity of lung ACE when compared with that obtained in the lung from normal rats. Addition of zinc ion to the superfusion medium did not alter the decreased conversion rate in the lung from zinc-deficient animals, indicating a possibility of a lower enzyme induction after

The effect of zinc deficiency on b.wt, zinc content of the bone as measure in femur and the conversion rate of A I to A II in the isolated perfused lung

	B.wt (g)	Zinc content of femur (µg/g wet wt)	Conversion (%) of A I to A II in the isolated perfused lung measure in rat ascending colon
Control rats in normal diet	201.0±5.0 (n=6)	189.2±6.6 (n=10)	26.8±2.5* (n=6)
Rats fed with Zn-deficient diet for 28 days	105.5±4.3 (n=10)	86.8±5.7 (n=10)	4.7±0.7* (n=8)

The animals were fed with zinc-deficient diet for 28 days (mean ±SEM). * $p<0.001$. Number of experiments in parentheses.



Isolated rat ascending colon superfused with the venous outflow of the isolated perfused rat lung. The perfusion flow was 10 ml/min and kept constant throughout the experiment. The response of the colon to the directly applied angiotensin II (A II) and angiotensin I (A I) was recorded. The response of the colon to A I was significantly potentiated when given through the pulmonary artery of the isolated normal rat lung (A); however, the response of the rat colon to A I given through the pulmonary artery of the lung from zinc-deficient rat was not significantly potentiated (B). The numbers on the bottom of the tracings indicate the concentrations of the peptides (ng/ml).

zinc deficiency. Zinc deficiency also causes a decrease in the activities of lactic dehydrogenase, alcohol dehydrogenase, alkaline phosphatase and some other enzymes[12]. In addition to these alterations, the present results clearly indicate that lung ACE also decreases in the zinc-deficient rats. Changing the zinc-deficient to the normal diet has been shown to cause a recovery in the decreased activity of the enzymes investigated[12,13]. Whether or not the same recovery also occurs in the activity of ACE is under investigation.

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A simple interspike interval analyzer for study of neuronal spike trains

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Summary. A simple, low cost interspike interval analyzer for the analysis of trains of nerve impulses is described. The analyzer is built with readily available integrated circuits and has been used to analyze spike trains in the lateral vestibular nucleus of cats.

Analysis of trains of nerve impulses of spikes is a common approach in the study of information transmission in the nervous system. That stimulus intensity is coded in terms of spike discharge frequency was first discovered by Adrian and Zotterman[2]. Since then the concept of a rate or frequency code in neuronal activities has been widely accepted. In 1946, the possible significance of the interspike interval in neural coding was first pointed out by Brink, Bronk and Larrabee[3]. Although a number of possible codes involving statistical analysis in the time and frequency domains have since been proposed[4,5], the interspike interval histogram has remained an important tool in attempts to decipher the code in impulse trains. We have designed and built a simple interval analyzer with LED dipslays which could also be linked to a X-Y plotter for instant plotting of data. The interval analyzer is easy to construct and consists of low cost integrated circuit components. The block diagram for the interspike interval analyzer is shown in figure 1. The essential components are a series of counters (SN 7490), random access memory chips (MM 2102), magnitude comparators (SN 7485) and a crystal oscillator.

The analyzer is governed by a sampling time control which determines the time during which analysis of the intervals is to be made. Preselected sampling time ranging from 10 to 90 sec or longer is derived by dividing a 1-kHz signal from an oscillator through a series of decade counters. Output from the sampling time control circuit is used to gate the input signals, i.e. nerve impulses, into a pulse counter. The pulse counter then totals the number of spikes that have appeared within the preselected sampling time. This number is instantaneously displayed. The nerve impulses are also fed in parallel to an interval counter, which is also driven by the 1-kHz oscillator and is capable of measuring intervals with an accuracy of $\pm$ 1 msec. The interval counter consists of a series of one-shot monostables triggered by each incoming signal. These monostables serve a dual function. They enable the memory units for storage of

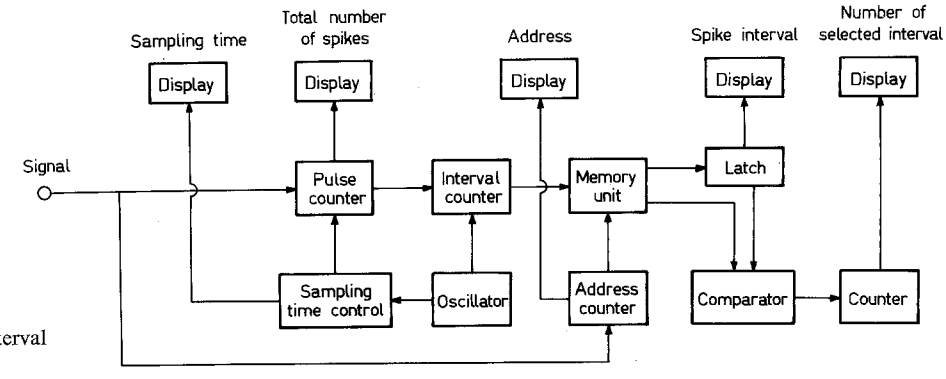

Fig. 1. Block diagram for the spike interval analyzer.