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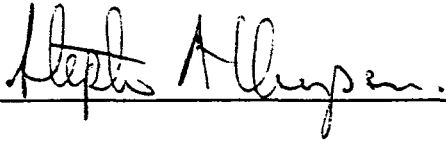
**Osmoregulation of system A amino acid transport  
in cultured mammalian cells**

Jie-Guang Chen

Submitted to the faculty of the graduate school  
in partial fulfillment of the requirements  
for the degree  
Doctor of Philosophy  
in the Department of Physiology and Biophysics  
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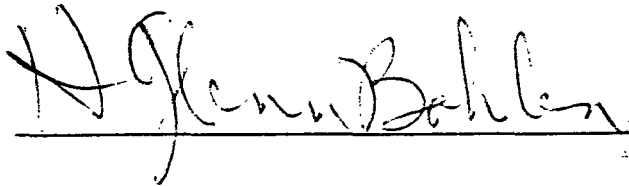
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Doctoral  
Committee



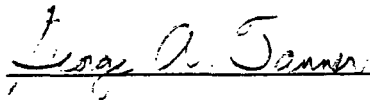
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Stephen A. Kempson, Ph.D., Chairman



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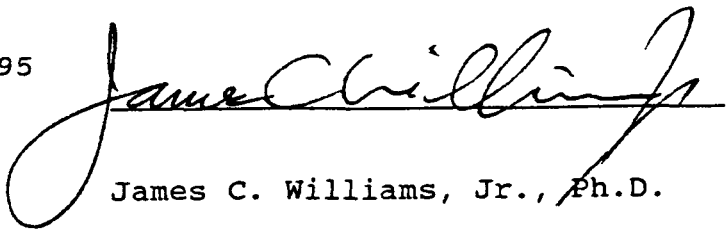
H. Glenn Bohlen, Ph.D.



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George A. Tanner, Ph.D.

May 23, 1995



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James C. Williams, Jr., Ph.D.

**Abstract**

During intestinal food absorption or renal concentration of urine, smooth muscle cells and epithelial cells in these tissues face osmotic stress due to the high extracellular solute concentration. When the stress is prolonged organic solutes (osmolytes) may be accumulated inside the cells to balance the extracellular solute concentration. System A, which transports neutral amino acids, is a sodium-dependent process that will bring amino acids into the cells to function as osmolytes. This thesis is focused on the response of system A to increased osmotic stress and the role of microtubules in this response. The contribution of system A to the cellular adaptation to osmotic stress was examined by comparing system A with the transport of betaine (another osmolyte) in renal epithelial cells. The main findings and conclusions are as follows:

1. Specific activation of system A transport occurred in response to prolonged hypertonic stress in both vascular smooth muscle and renal epithelial cells. Both hypertonic activation and isotonic recovery of system A required new protein synthesis. Hypertonicity, by increasing the intracellular ionic strength, may disrupt a system A repressor and so increase the expression of a system A related protein.
2. Microtubule disruption by various drugs in serum-free culture medium stimulated system A in smooth muscle cells. This stimulation required de novo protein synthesis and had no additive effect with the serum stimulation. This stimulative effect compromised

the requirement of intact microtubules for the hypertonic activation of system A. However, in the presence of serum or higher osmotic stress, microtubule disruption partially prevented the hypertonic activation of system A.

3. In renal epithelial MDCK cells system A was activated and reached peak after 8 h of hypertonic stress. After 24 h system A was recovered close to its initial value. This later downregulation was accompanied with the activation of specific betaine transport, suggesting that system A<sub>1</sub> may provide an early temporary relieve by accumulating neutral amino acids, for these cells under the hypertonic stress.

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