

# This Week's Citation Classic®

Gruber K A, Whitaker J M & Buckalew V M, Jr. Endogenous digitalis-like substance in plasma of volume-expanded dogs. *Nature* 287:743-5, 1980.

[Departments of Physiology and Pharmacology, Medicine and Microbiology and Immunology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC]

This paper provided evidence for an endogenous circulating "digitalis-like" substance. Extracts of dog plasma previously shown to contain natriuretic hormone-like activity were also found to inhibit Na,K ATPase activity and possess antidiuretic immunoreactivity (i.e., digitalis-like properties). Increased amounts of digitalis-like activity were detected in dog plasma during extracellular fluid volume expansion, suggesting that the putative natriuretic hormone may have digitalis-like properties. [The *SCF*<sup>2</sup> indicates that this paper has been cited in more than 380 publications.]

## An Endogenous Digitalis-Like Substance

Kenneth A. Gruber

Division of Communication Sciences  
and Disorders

National Institute on Deafness and Other  
Communication Disorders  
Rockville, MD 20853

My interest in natriuretic hormone (NH) began in 1975 when I was a postdoctoral fellow in Sidney Udenfriend's group at the Roche Institute of Molecular Biology. Neil Bricker came to us for help in purifying natriuretic activity from uremic urine, which he thought was attributable to a small peptide.<sup>1</sup> The inconsistent chemistry of Bricker's urinary extracts convinced me that a better starting material was needed, and I thought that plasma might be the answer.

My acceptance of a position at Wake Forest University led to a collaboration with Vardaman M. Buckalew, who had worked with natriuretic plasma extracts for some time. Our first publication presented evidence for a circulating precursor of NH and the first isolation of the activity on high performance liquid chromatography.<sup>2</sup>

It was soon clear that the amount of material needed to perform biological assays was so great that it precluded the monitoring of chemical isolation steps without considerable losses. A then-emerging concept in the NH field was that its mechanism of action was through inhibition of renal Na,K ATPase,<sup>3</sup> i.e., an ouabain or digitalis-like action. I recalled some work by Sidney Spector that antibodies to a given drug might also function as a surrogate

receptor for endogenous substances with similar biological activity. I speculated that if NH had some of the biological properties of the cardiac glycoside class of drugs, it might also cross-react with antibodies to a cardiac glycoside.

Buckalew felt that it was a "wild hypothesis," but it was also too easy an experiment not to do. We purchased a clinical digoxin RIA kit, tested our extracts, and were somewhat amazed to find that it was relatively easy to demonstrate endogenous digoxin immunoreactivity. As we later discovered, our work resurrected and expanded earlier observations of "apparent" digoxin immunoreactivity in patients with cardiovascular or renal disease.<sup>4</sup> In the ensuing years, many investigators have tried to prove that certain known endogenous substances can account for endoxin. However, none of these substances appear to exhibit the relationship to cardiovascular parameters which both we and S.W. Graves et al.<sup>5</sup> described.

Interestingly, the original abstract describing endogenous digoxin immunoreactivity was rejected for presentation at the American Society of Nephrology meeting in 1979. In the ensuing years, virtually all the society's reviewers have approached one of us to deny that they were the ones who rejected our abstract. I can therefore only assume that the rejection was a "clerical error."

During *Nature's* review of our manuscript, we learned that another group at Harvard was trying to search for a circulating digitalis as well. Indeed, as we later discovered, one of these investigators was a reviewer for our paper. It took almost a year to get our report accepted by *Nature*. After the second submission, one of the reviewers tried to change the requirements for acceptance he had laid down in his first review. Fortunately, the editors would have none of that!

One of the immediate honors resulting from this work was a Research Career Development Award from the National Heart, Lung, and Blood Institute. This gave me the freedom to travel and work in other laboratories in the US and Europe, leading to friendships that continue to this day.

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