Discussion

DR. JOHN B. HANKS (Charlottesville, Virginia): This is another elegant study by Courtney Townsend's group and I'm flattered to be asked to comment on the manuscript. Dr. Townsend and Dr. Thompson have both pioneered the novel concepts of GI peptides' possible regulatory effects on GI tumors. No laboratory in the world has produced the findings that they have had and, according to the old adage, really nobody does it better. This elegant paper supports the concept that certain administered doses of neurotensin stimulate the growth of the cell line of a known pancreatic cancer paralleling the same concept that this group has reported studying gastrin's effect on known gastric cancer. There is still obviously much work to be done to reconcile some of the disparate findings of other groups. For example, an article has appeared recently by Tatsuda's group in Japan which presents evidence that neurotensin treatment of Wistar rats using an azaserine-induced pancreatic acinar tumor line really has resulted in inhibition of pancreatic carcinogenesis. Other groups have demonstrated that neurotensin secretion exists in certain pancreas endocrine tumors including the Zollinger-Ellison syndrome. Much work clearly remains to be done to sort out the possible connection between a peptide which is secreted largely in the distal terminal ileum and colon and its possible stimulatory role in pancreatic carcinogenesis. I have a couple of questions for Courtney. Can you expand on the *in vivo* observations or clinical applications to your findings? The cell line data are compelling, but what evidence occurs that you found or how are you going to move into the intact organism and demonstrate that carcinogenesis occurs at the physiologic levels of peptide secretion that you've worked with? And do you have any data that increased levels of neurotensin exist in patients with pancreatic carcinoma, or conversely, that increased neurotensin secretion in the endocrine tumors might be associated with colon carcinoma? The second question would be that if neurotensin is indeed a carcinogenic agent, can you hypothesize, and you spoke about it a little bit with your last slides, but can you hypothesize that a neurotensin antagonist which would take the role of a calcium-blocking agent or a protein kinase C suppressor, would be an effective chemotherapeutic treatment for pancreatic carcinoma?

DR. DAVID S. ROBINSON (Miami, Florida): I want to congratulate the authors on a very elegant study that not only demonstrates the observation of mitogenesis in pancreatic cancer but also starts to look carefully into its mechanism. I have several questions for you, Dr. Townsend. First, what are the levels of neurotensin in fetal calf serum and bovine serum albumin? You have used 5% levels of calf serum in your culture medium, .5% in your wash solution, and .1% bovine serum albumin in wash solution. Do neurotensin levels here have any impact on the study? Second, you suggest an alternative postulate, that there might be two clones of cells, one reacting to the high affinity receptors and one to the low affinity receptors. Have you, in fact, subjected this hypothesis to a single cell cloning assay to disprove that possibility? Also would you give us further thoughts on how crosstalk works? It is not entirely clear to me what the implications are. What further studies have you developed to investigate this area? What are the physiologic levels of neurotensin in man both serologically and at tissue level? Finally, this begs the question, is the observation of mitogenesis by neurotensin significant for *in vivo* pancreatic cancer or is it simply an interesting but not applicable laboratory study? You alluded to the possibility that these findings may lead to a strategy in the treatment of pancreatic carcinoma. Based on the early observations, how would you proceed with an approach that would come to an *in vivo* analysis and eventually to a clinical trial?

DR. COURTNEY M. TOWNSEND, JR. (Closing Discussion): I'll answer the questions that are different specifically and then as some of the questions have a common thread, then I'll try to put those together. Dr. Hanks asked about the evidence in rats treated with azaserine that neurotensin may, in fact, be associated with decreased development of tumors. Azaserine in rats produces tumors of the acinar cell type that are not related to ductal cancers. Human cancers are ductal and the only animal model for ductal cancer is the Syrian golden hamster treated with nitrosamines. I don't know how to explain the findings of Tatsuda in rats. I know of no information on carcinogenesis in the ductal model. The levels of neurotensin, both questioners asked about those. Physiologic levels of neurotensin are achieved in patients that can be measured by radioimmunoassay after a meal or after fat. The signal comes from the proximal gut and is not translated if fat is placed in the distal gut. And we don't know of any instance in which increased levels are noted in patients with pancreatic cancer or conversely after operation whether there is any change. We've not studied those. Tumors that produce neurotensin are known, largely functional islet cell tumors. No known syndrome of a neurotensinoma has yet been described. They are most often part of polyhormonal elevation in the face of a syndrome due to another agent. And I don't know of any instance in which any increase in instance of cancer of the gut or the pancreas has been described. Dr. Robinson asked about neurotensin in the medium and in the bovine serum albumin. We've been unable to measure any levels of neurotensin in those agents that we use, and again the serum concentration in the test agent is either .5 or .1%. The question about whether we're seeing response of two clones of cells as opposed to two receptor types on the same cell. We've not yet been able to establish stable clones that exhibit different properties and so have not yet been able to answer that question. In terms of future strategies to use knowledge that we have gained about the mechanisms by which hormones affect tumor growth, it would have multiple levels. We could interact with the agent at the receptor site, we could prevent the release of an endogenous agent, or we can block various steps along the pathway or pathways with specific agents to inhibit the effect of that hormone. Those combined with other strategies, the use of cytotoxic agents or other agents that work through separate pathways, we hope then would allow potentiation and decrease tumor growth to occur. We have begun those kinds of studies, not with neurotensin in pancreatic cancer, but have shown that there is significant potentiation. For example, we used somatostatin combined with alfa interferon and an inhibitor of polyamine biosynthesis DFMO in the growth of human carcinoid cells, growing in nude mice.