Informal Discussion following the Paper by Longnecker and Curphey

Dr. Pledger: With your azaserine- and alanoser-treated animals in which you did detect foci of atypia in the pancreas, did you find such lesions in any other tissues? Did you actually look for any alterations in other organs, such as the kidney for example, where the compounds do concentrate at a significantly higher level?

Dr. Longnecker: We always take a section of kidney and we always examine the kidney grossly. When we see no gross lesions we take a routine section, but we do not totally embed the kidney. We have not seen any change in the kidney. At this time we have seen changes only in the pancreas.

Dr. Sasmore: I was struck by the possible similarity of the cell type to some of those that we may have observed in the previous presentation. You were able to demonstrate that these were of acinar origin. My question would be: if these were permitted to go to termination, might they so alter the pancreas that the zymogen granules would no longer be identifiable? To put it another way, is it possible that some of the tumors that were presented in the human cases would have indeed been able to present zymogen granules had such tissue been available for examination earlier?

Dr. Longnecker: Well, I think we can't answer that; I see an overlapping spectrum of histology. However, our *in situ* stage, if we can regard the nodules as that, are clearly in the acinar cells and not in the ducts, and we have seen no changes in the ducts, similar to those that Dr. Fitzgerald illustrated. Terminally, late in the stage of the disease, some of the tumors are poorly differentiated; and, I think, at that stage it is really hard to say what the cell of origin was. It is the evolution of changes in a group of animals that makes us think it is primarily in the acinar cell.

Dr. Weisburger: Two questions, Dr. Longnecker, which I find of interest. Did you have any success in localizing azaserine or metabolites within the pancreas head and tail? Secondly, does azaserine in any way affect the function of the acinar cell biochemically, especially early in the test?

Dr. Longnecker: In terms of the site of tumors we have seen, the nodules are obviously diffuse throughout the pancreas. We've seen malignancies in all regions, but I guess we really haven't done a definitive breakdown in terms of head *versus* tail. With respect to your 2nd question, we have not really studied the effect of azaserine acutely on the pancreas. I think there is reason to assume that it interferes with DNA synthesis, because this is well worked out in other tissues.

Dr. Pienta: We have examined azaserine and 6 of the nitroso derivatives prepared by Dr. Curphey, and we find that 2 of these, alanoser [*i.e.*, *O*-(nitroso-*N*-methyl-β-alanyl)-L-serine] and *N*'-(*N*-nitrososarcosyl)-L-lysine, were able to transform early passage hamster embryo cells *in vitro*. We also found that cells treated with azaserine have the ability to grow in semisolid agar, which is 1 criterion for transformation. These cells also induce tumors in nonimmunosuppressed hamsters when injected s.c. at a million cells per animal.

Dr. Longnecker: When we gave up attempts to utilize the hamster cell culture system, we were happy to have Dr. Pienta consent to do these studies with the compounds in which we have had interest. And, I think ultimately it will be of interest to correlate the animal, bacterial, and cell culture studies.

Dr. Tannenbaum: I was interested in your mutagenicity test with the Ames system. You didn't mention whether or not you used metabolic activation.

Dr. Longnecker: It was without activation.

Dr. Tannenbaum: I wondered why you would have expected alanoser to be active without metabolic activation.

Dr. Longnecker: We didn't.

Dr. Pienta: When tested against hamster embryo cells, alanoser does transform without adding liver microsomes for metabolic activation in the hamster cell system.

Dr. Barron: You indicated the distribution of these chemicals in the various organs, but in what cell were they concentrated, and where in the different cells?

Dr. Longnecker: We don't know the answer to that question. These studies were done with tissue homogenates, and we are making the assumption that biochemists have been making for years, *i.e.*, that in the pancreas this probably primarily reflects the acinar cell. We have not done radioautographic studies and really don't have the definitive data.