See re-typed version & I returned — Sept 6

Heidelberg Plenary Lecture Tuesday August 30, 1994.

Angiostatin: A novel protein by which primary tumors suppress growth of metastases.

Thank you (We're) Dr. Rosau

This well organized meeting that ~~D. Rosau~~ Werner Risau and his colleagues have assembled with its many very exciting titles to be presented, symbolizes one of the unique distinctions of the field of *vascular biology* in general, and of angiogenesis research in particular: . . . . there is an immense collegiality among ~~our~~ the many researchers in these fields.

Despite an imposed competition for grants, and other impediments, people in this field come to the meetings, like this not to find out who is ahead of whom / or who just cloned the latest gene ahead of others, but to learn **how** certain central questions in the field are being attacked, or **if**, last year's mystery is still a mystery! [Example, How are signal-less peptides exported? Does VEGF have responsive receptors on cell types other than endothelium?] etc.

And one will hear in the coffee breaks and at meals, . . . a certain shared enthusiasm for someone else's new finding: "A ha! So that's the explanation!" ~~One finds~~ There are few scientific fields with the *comraderie* of this one, ...with the possible exception of those happy souls who study **C. elegans**, and chatter to each other in their weekly "Worm Lover's Journal," about their latest discoveries. (or the ~~Drosophila~~ Drosophila scientists)

So, in that spirit, I wish to tell you about a new finding, ~~in fact on the eve of its publication~~, . . . one that I began to think about 4 years ago, followed by 3 years of experimental work with **Michael O'Reilly** who came as a post-doc. I presented the outlines of this work in an unfinished form, at a Keystone meeting last February, and now wish to show you how it turned out.

Virtually all solid tumors are angiogenic and neovascularized by the time they are detectable in animals and humans. But, when they first originate, most tumors are not angiogenic, and in humans, carcinoma *in situ* ~~is~~ may be not angiogenic for months to years!

Thus, a central problem in tumor growth is how tumor cells switch to the angiogenic phenotype!

In the early 1980s, when the first POSITIVE regulators of angiogenesis were purified, it was thought that the "switching problem" could be understood solely in terms of the behavior of these polypeptides, such (FGF, and later VEGF, etc).

For example, If one just knew enough about how bFGF was exported out of cells etc, one would eventually know how tumors switched ON angiognesis ~~and kept it ON, when normally it was such a rare and~~

Slide 34: (diagram of sequence)

Microsequence analysis revealed that the protein is homologous to an internal fragment of **plasminogen**, specifically a fragment of the A-chain of plasmin with an N-terminus at amino acid 98 and a carboxy terminus at approximately amino acid 440.

-(This fragment inhibits endothelial cells, but full-length plasminogen does not). We named it "**ANGIOSTATIN**").

[These results were obtained for 2 separate purifications by N-terminal analysis and by analysis after tryptic digest, by Bill Lane at Harvard's dept of biochemistry)

Slide 35: Angiostatin includes the first 4 triple loop structures, or "kringle" regions, of plasminogen].

[V = valine; Y = tyrosine; L = leucine; S = serine; E = glutamine; X = ? K = lysine].

Slide 36: (Commercial human plasminogen on SDS-PAGE)

When commercially available **human** plaminogen fragments were purified, capillary inhibitory activity was found again in a region (generated by elastase) that included the first three Kringle structures.

When this was purified by several cycles of HPLC, it revealed 3