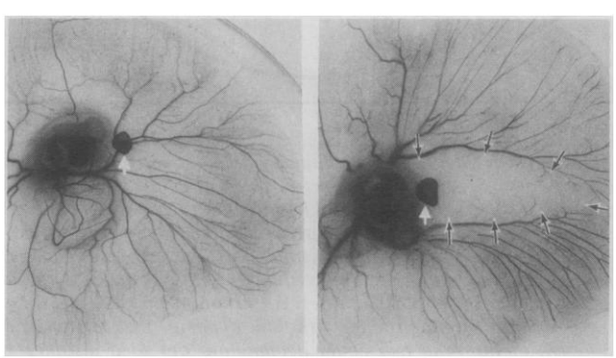
New Angiogenesis Inhibitor Identified

The first characterization of a specific inhibitor of angiogenesis, the process of recruiting new blood vessels, has been reported by Judah Folkman and Stephanie Taylor of the Children's Hospital Medical Center, Boston, in the 27 May issue of *Nature* [**297**, 307 (1982)]. Angiogenesis plays a key role in the growth of tumors, in diabetic retinopathy, and in retrolental fibroplasia (in which blood vessels proliferate in the eye), and perhaps even in arthritis (*Science*, 19 June 1981, p. 1374). The new inhibitor will probably not be useful for therapy of these conditions, but it should provide a valuable tool for studying the mechanisms, and could point the way toward development, of better therapeutic agents.

Angiogenesis inhibitors have previously been isolated from cartilage and from the vitreous humor of the eye. These inhibitors are proteins that bear an unusually large number of positive charges, but they have not yet been further characterized because they are available only in extremely limited quantities. Promoters of angiogenesis have also not been characterized for the same reason. The new inhibitor, in contrast, is a commercially available product whose structure is known. Its availability should provide a major breakthrough in the study of angiogenesis.

The inhibitor is protamine, an arginine-rich (and thus positively charged) protein found only in sperm. There are actually several different protamines isolated from different species. Taylor and Folkman used a product obtained from salmon sperm. The principal constituent of this protamine has been purified and its sequence has been determined, but the commercial product they used is actually a mixture of several related proteins with an average mass of 4300 daltons. They found that this substance inhibits the capillary proliferation observed in the growth of embryos, in inflammation and certain immune reactions, and in the growth of solid tumors.



Judah Folkman

A new angiogenesis inhibitor

Protamine in a slow-release pellet (right) blocks the growth of new blood vessels toward a tumor implanted in a rabbit cornea, while the pellet alone (left) has no effect.

The discovery of protamine's inhibitory effect was serendipitous. In their earlier studies of angiogenesis, Folkman and his colleagues had observed that mast cells—formative cells of connective tissue—accumulate at a tumor before growth of new capillaries begins. These cells alone, however, cannot initiate angiogenesis. Folkman's group found that the mast cells at the tumor site release heparin, a mucopolysaccharide with well-known anticoagulant activity. The heparin apparently increases the migration of capillary endothelial cells toward the tumor, a first step in the growth of new blood vessels.

Heparin alone is also not angiogenic, but it potentiates the activity of angiogenesis promoters. In embryonic growth studies of the chick chorioallantoic membrane, for example, Taylor and Folkman found that the combination of heparin and a small quantity of an angiogenesis promoter isolated from a tumor had the same effect as four times as much promoter in the absence of heparin. Folkman terms the discovery of this activity of heparin as important as the discovery of the inhibitory activity of protamine.

The pair began using protamine because it is known to bind heparin. They found, in fact, that a small quantity of protamine tied up the heparin and reduced angiogenic activity to the level that would be expected in the absence of heparin. The addition of more protamine, however, completely blocked angiogenesis. A similar effect was subsequently observed with tumors implanted in the cornea of the rabbit eye and in the vascular bed of the rabbit ear—both standard assays for determining angiogenesis activity. Protamine also blocked the stimulation of capillary growth normally observed when lymph node fragments are implanted in the cornea.

The availability of significant quantities of protamine made it possible for the first time to study the systemic administration of an angiogenesis inhibitor. Unfortunately, protamine produces lethargy, weakness, and, occasionally, sudden death in laboratory animals if given in high doses. Nonetheless, the substance produced a 77 to 97 percent inhibition of lung metastases in mice. It also inhibited the growth of a tissue culture strain of B16 melanoma injected subcutaneously in mice, but it had no effect on two other types of tumors injected subcutaneously.

In control studies, Taylor and Folkman found that protamine is not toxic to tumor cells growing in culture or to rapidly dividing cells in mouse bone marrow. This and other evidence indicates that the substance exerts its antitumor effect through its influence on angiogenesis. They also studied related compounds, such as poly-L-arginine, poly-L-glutamic acid, and poly-L-lysine. None of these had any effect at concentrations two to four times that of the maximum tolerated dose of protamine, but poly-L-lysine inhibited angiogenesis in the chorioallantoic membrane weakly at five times the protamine concentration, a near toxic level.

Angiogenesis inhibition is a newly found property of protamine, but antitumor activity is not. Protamine was used in England in the early 1960's to treat breast cancer and certain other tumors in mice and humans. The British investigators found that the tumors regressed during treatment with protamine, but resumed growth when the treatment was stopped. They also observed cumulative toxicity in humans. But if protamine can be used to determine the mechanism of angiogenesis, says Folkman, it should be possible to design angiogenesis inhibitors that are both more potent and less toxic. These could be especially effective when used in conjunction with conventional chemotherapeutic agents.—THOMAS H. MAUGH II

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