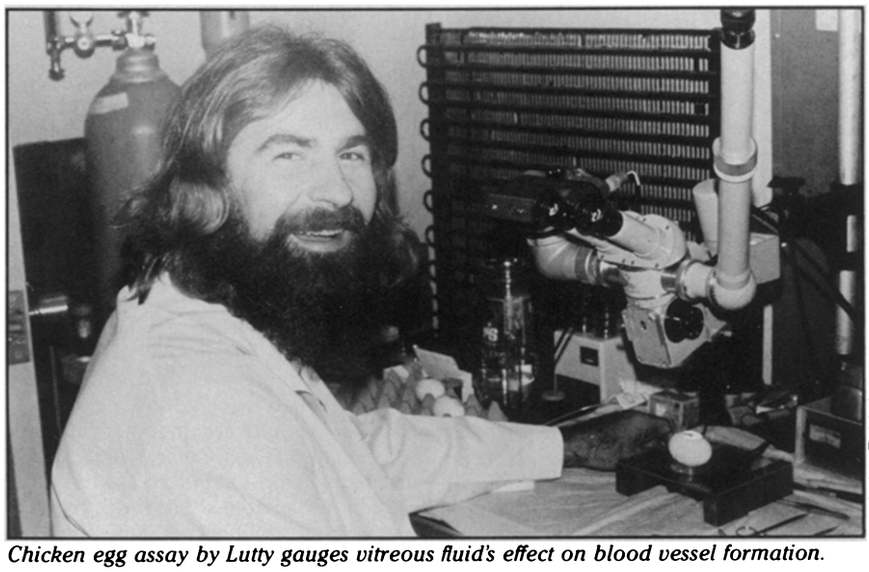
Don't Feed the Tumors

Cartilage and vitreous fluid both contain chemicals that inhibit new blood vessel formation. Research on these chemicals may help in the fight against two blood vessel-dependent diseases — cancer and diabetic retinopathy.

BY JOAN AREHART-TREICHEL



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Chicken egg assay by Lutty gauges vitreous fluid's effect on blood vessel formation.

Tumors don't just sit there. They take an active part in ensuring their own survival. For one thing, they stimulate the development of new blood vessels in host tissue and then use those vessels to get the nourishment necessary for growth. In 1969 Harvard Medical School surgeon Judah Folkman found that tumors produce a substance that stimulates blood vessel growth. He called the substance "tumor angiogenesis factor," or TAF (angiogenesis means "creation of blood vessels"). Since then he and his co-workers have found that most tumor cells produce TAF and that without it tumors cannot grow larger and spread.

If a way can be found to stop angiogenesis, it might lead to treatment strategies not only for cancer but for diabetic retinopathy, the major form of blindness in the United States among persons older than 60 years of age. The pot of gold at the end of this rainbow is not yet in sight, but research by Folkman and others may be leading in the right direction.

After Folkman discovered TAF and began to understand its implications for cancer growth, he and medical student Henry Brem mulled over ways to thwart TAF and thereby inhibit cancer. For instance, could they block the production of TAF by tumors? Could they keep TAF from reaching blood vessels? Could they prevent TAF from attaching to blood vessel receptors? None of these strategies was possible at the time because there was no purified form of TAF and because there was no way to isolate blood vessel receptors. Taking another tack, they began looking for some way of keeping blood vessels from growing into tumors.

First Brem learned that fetal cartilage is vascularized but later, in adult form, contains no blood vessels, suggesting that it might, in its early development, produce some chemical that causes regression of blood vessels. Then Folkman and Brem heard that Reuben Eisenstein and Klaus Kuettner of the University of Chicago Medical School had just found that cartilage does contain chemicals that keep blood vessels from growing into it — further evidence that cartilage contains some molecule or molecules capable of preventing angiogenesis. Folkman and Brem then tested the possibility that cartilage, or compounds in cartilage, might keep blood vessels from growing into a tumor. They implanted a tumor into the cornea of the eye of a rabbit, then placed cartilage from a newborn rabbit next to the tumor. Sure enough, the cartilage kept blood vessels in the cornea from growing into the tumor. So some chemical or chemicals in cartilage had ability to combat tumor growth by keeping blood vessels from making contact with the tumor.

The next challenge was to purify the cartilage chemical or chemicals capable of such a feat. Folkman and Brem managed to partially extract one chemical with the help of Robert Langer, assistant professor of nutritional biochemistry at the Massachusetts Institute of Technology. The crudely purified compound was able, in rabbit corneas, to inhibit blood vessel contact with tumors as well as inhibit tumor growth, just as cartilage itself could. The researchers then found that they could inject the chemical into rabbits' bloodstreams and get the same results with no detectable toxicity.

Meanwhile, a Baltimore scientist entered the picture and added still other exciting possibilities — that the cartilage chemical, or something like it, might be able to ward off the blood vessel proliferation of diabetic retinopathy as well as of cancer. He was Arnall Patz, an ophthalmologist at the Johns Hopkins Medical Institutions.

In the 1940s blindness had been rampant among premature infants whose lives had been saved by incubators. It was Patz who found the reason: The incubators were designed so that too much oxygen reached the preemies. Once this problem was corrected, a major cause of blindness in the United States was eliminated, and Patz received many honors for his discovery, notably a Lasker Award in 1956. Patz later turned his interests to what is now one of the major forms of blindness in the United States — diabetic retinopathy, which is caused by proliferation of blood vessels into the retina of the eye. Patz and Johns Hopkins ophthalmologist Daniel Finkelstein, having heard about the research of Folkman, Brem and Langer, thought that TAF could be made to produce an animal model of neovascularization similar to that in retinopathy (in other words, could stimulate proliferation of blood vessels of the retina upon being injected into various areas of an animal's eye).

Patz and Finkelstein were then joined by Steven Brem, a Boston neurosurgeon. They obtained some TAF from the Harvard scientists and tested the hypothesis. It was borne out, but with one unexpected exception: When TAF was put in the vitreous fluid of the eye, it did not stimulate proliferation of blood vessels of the retina. The researchers hypothesized that a chemical in the vitreous fluid, as in cartilage, was keeping TAF from stimulating blood vessels and was preventing blood vessel proliferation.

The collaborators then found that a crude extract from normal vitreous fluid could inhibit blood vessel growth. Gerard Lutty, Patz and co-workers at Hopkins further purified the material from vitreous fluid and implanted it in a rabbit's cornea rather than in a rabbit's retina because it was easy to see and measure blood vessel

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growth within the transparent cornea. Again they found that the vitreous fluid chemical, like the cartilage chemical, was able to keep the blood vessels from contacting the tumor. Allan Fenselau, a biochemist at Hopkins, meanwhile had developed a cell culture system with aortic endothelial cells, the major cell type in blood vessels. The vitreous inhibitor was found to prevent the growth of the endothelial cells in this system without damaging them.

But now still another provocative question surfaced: Is the vitreous inhibitor the same as the cartilage inhibitor? To date, Folkman, Henry Brem and Langer are still not sure whether their inhibitor (taken from calves) is a protein, although they have a hunch it is. The Hopkins team is relatively certain that their inhibitor is a large protein that is very stable, since it resists boiling. How long will it take the Harvard and Hopkins scientists to thoroughly purify and identify their inhibitors? Langer replies: "That is a hard question on which to make a prediction. The animal assays we use are difficult and also take a lot of material." Fenselau concurs. So does Brem: "We never know what roadblocks lie ahead. Every one of the steps so far could have taken 10 years."

Even assuming that the two inhibitors are purified and identified within the next five years, the challenge remains of getting enough of the chemicals for animal and clinical tests. (It now takes literally tons of cartilage to test the cartilage inhibitor in a few rabbits.) But, assuming that's possible, the ultimate challenge lies in seeing whether the inhibitors can alter blood vessel proliferation not only in solid tumors but in the retinas of patients with diabetic retinopathy. The vitreous inhibitor might also be tested on brain cancer patients, because brain tumors are the most vascularized of all tumors. And while laser photocoagulation can now seal off damaged, proliferated blood vessels in the retina and prolong the sight of diabetic retinopathy victims (SN: 4/10/76, p. 232), the inhibitors might turn out to be superior to photocoagulation treatment if they prevent blood vessel proliferation before it actually takes place.

And of course, assuming that the inhibitors turn out to be valuable treatments for cancer and diabetic retinopathy, they would still have to be thoroughly tested before the Food and Drug Administration would put them on the market. But would there be enough of the inhibitors for marketing? If they prove to be proteins, perhaps protein chemists could synthesize copies of the real stuff, or the protein could be mass-produced with recombinant DNA techniques — altogether new challenges in their own right. So the road is still a long one, but the researchers involved are optimistic about TAF inhibitors. Says Patz: "I'm encouraged about the possibility that they might ultimately have an application." □

. . . Fractals

Coastlines, river systems, islands and seas, mountains and lakes are all well modeled by different kinds of fractals. In Voss's analysis a musical composition becomes something of a linear mapping problem.

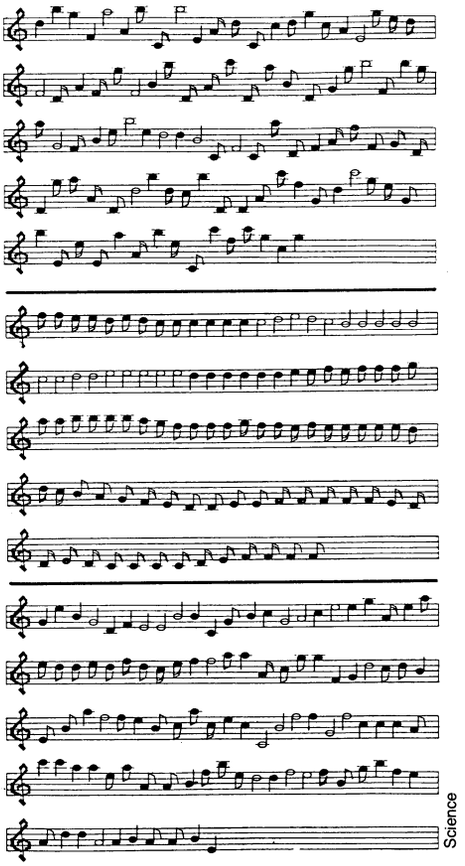
The other element in Voss's analysis is $1/f$ noise. This is a phenomenon long familiar to electronics engineers, although they are not likely to have considered it music. Information theory distinguishes three kinds of noise according to how the intensity of the sound at any given frequency depends upon the value of the frequency. They are white noise, in which there is no frequency dependence, a totally uncorrelated random spectrum, $1/f^2$ noise, which is related to Brownian motion and is well correlated and well understood, and $1/f$ noise, which is intermediate between the two, more correlated than white noise, less correlated than Brownian motion.

$1/f$ noise appears in numerous and varied natural phenomena. It first came to attention in the output of old-fashioned carbon microphones, but it occurs in all electronic components. It limits the accuracy with which time can be measured in instruments from hourglasses to crystal oscillators. Time correlations of this sort are found in undersea currents, Nile flood levels for a thousand years, ionic transport across nerve membranes. There is "no universal explanation for its occurrence," says Voss. And he poses a philosophical question about how music interacts with nature, whether "music imitates the way our world is changing in time."

An analysis of the sound spectrum of J. S. Bach's First Brandenburg Concerto fails to show any $1/f$ correlations. Yet you would expect them somehow in music, says Voss, because the structure of music is fractal, being divided into bars and sections hierarchically organized, being often selfsymmetric. $1/f$ noise has fractal relations, too. To get the formula for $1/f$ noise mathematicians divide the frequency range into short sections like musical bars and relate the sound intensity over each bar to the place of that bar in the succession of bars.

Examination of both loudness fluctuations and melodic variations yields success. Voss says he listened to a classical station, a rock station and even a news and talk show. Melodies from all over the world were checked, too. "There is really no difference between the Beatles and Beethoven," Voss says. "$1/f$ is the common element in music." Music from all over the world.

If this is so then it should be possible to take $1/f$ and map it back into recognizable music. First Voss and his co-workers checked music written by a random number generator. This sounds passing strange and looks weird on a score. It sounds strange because it has no time correlations. "Like monkeys banging on pianos," Voss says.



Machine-made music based on random tone generation (top), $1/f^2$ noise and $1/f$ noise. $1/f$ seems most like real music.

For a second trial they decided to give it some real correlation and used what mathematicians call a Markoff process to generate imitations of a set model. The model was a bit of melody written by the sentimental composer Stephen Foster. The idea was to generate a song Foster might have written. The process did all too well. It kept coming up with songs Foster actually had written.

The monkeys were totally uncorrelated, and the Markoff process altogether too correlated. That convinced Voss that down the middle with $1/f$ was the way to go. He chose Gregorian chant for the first try because it has a very clean fractal structure, a triadic structure based on the Trinity. The idea was to generate short segments with $1/f$ time correlations and then map them into themselves with this triadic structure. The process generated a fake Gregorian chant that sounds like the real thing. It sounded surprisingly similar to a real one also exhibited.

Voss remarks that he did a similar thing with a piece of Chinese music and then played it for some Chinese students at Berkeley. They declared that it sounded familiar but not quite like what they heard at home — a little Vietnamese, perhaps. Voss thinks this is not bad. The fake music sounded not quite like the home product, but very close. So it seems to be upward and onward for composition by the $1/f$ plus fractal method: to multiple voices, polyphony, counterpoint, someday perhaps a tone poem on the fluctuations of the Nile. □