Proteins and Metals Stick Together

How a Nobel laureate solid-state physicist discovered a way of doing immunology by dunking

by Dietrick E. Thomsen

"Protein sticks to everything but itself," says Ivar Giaever of General Electric Laboratories. There is one exception to that rule: Antibodies will stick to the antigens they are coded for and vice versa. Giaever has used the rule and the exception to develop a method for immunological testing that he hopes will find clinical use.

Giaever won the 1973 Nobel prize in physics for his work on electron tunneling in thin films, but in recent years he has been into biophysics. The new work has a thread of a connection with his previous activities: It still concerns thin films, but it now deals with their biological interactions.

Giaever started into biophysics with a fascination for protein structure. He cites hemoglobin as an example. Proteins are polymers, and the monomers of which they are made are amino acids, of which about 20 kinds are known. Hemoglobin consists of four chains of amino acids, about 150 of them. Yet one single error in this structure can cause sickle-cell anemia. "I got interested," he says. "You can't cure sickle-cell anemia unless you do genetic engineering."

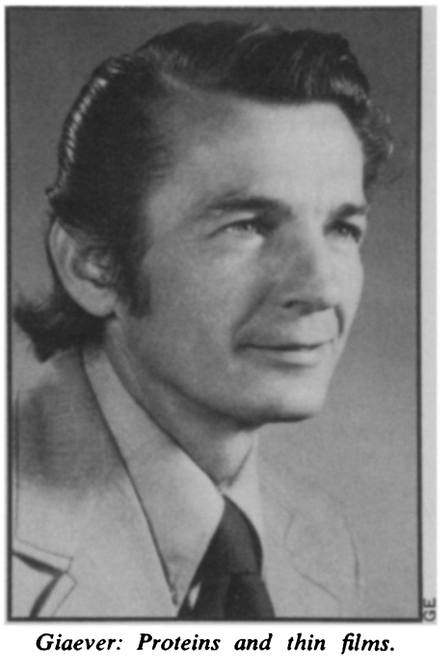
For the study he wanted to do, he had to commission the construction of a special microscope. While that was under way, he decided to "find out how protein acts on a metal surface." Such a study could be relevant to preparation of slides for the microscope. It was in this endeavor that he made his immunological discovery.

In what is standard procedure for a scientist entering a new field, he looked for literature on what had been done in the past. "There was no literature so I decided to find out myself," he says.

Experimentation started very simply with a tank of saline soluiton, a thin film of nickel deposited on a glass slide and a jar of bovine serum albumin (BSA). BSA is a protein derived from the blood of cattle and is a common substance for experimental use in laboratory experiments in biology.

The slide was immersed in the tank, and BSA was dribbled into the solution. The object was to see if and how the BSA might attach to the nickel surface. The BSA did, and Giaever was able to measure the thickness of the absorbed layer with a device called an ellipsometer, which uses polarized light. If plane-polarized light is reflected from such a surface, it will come off with its polarization changed to elliptical. By measuring this change with a photometer, one can calculate the thickness of the protein layer to a fraction of an angstrom. The measurement showed the BSA layer on nickel to be 30 angstroms thick. This is the size of the BSA molecule, and it indicates that the BSA layer was a monolayer, one molecule thick. No more BSA would stick.

Then Giaever decided to see if any other protein would stick onto the BSA. He took a slide with a layer of BSA stuck on, immersed it in a clean tank and dribbled rabbit serum into the tank. Rabbit serum contains 100,000 kinds of protein. Will any rabbit serum protein attach to the BSA? he asks. "You drop it in, nothing happens," he replies. "Protein sticks to everything but itself and other protein."



Giaever: Proteins and thin films.

But there is the one exception, as he discovered when he used serum from rabbits that had been exposed to BSA. It is here that the immunology comes into it.

We are dealing here with what is called humoral immunity, the kind of immunity that is mediated by substances in the blood, not the direct cellular immunity that is responsible for the rejection of transplants. Humoral immunity comes into play in response to virus diseases for example.

Suppose, says Giaever, that a virus of chicken pox or syphilis gets into the blood. ("I decide whether to talk about chicken pox or syphilis according to the age of the audience. I guess I had better talk about chicken pox today.") Such a foreign body is called an antigen, and the blood makes antibodies in response to its presence. Each antigen has its own specific antibody. The antibody goes and sticks to the antigen, thus labeling it as a foreign substance for the white blood cells. The white cells then come and eat up the whole thing.

So Giaever decided to see if he could get an antibody to BSA and see if it would stick to BSA on the metal film. The antibody is obtained by injecting BSA into the blood of a rabbit. "The rabbit thinks it's sick," says Giaever, "but it really isn't." Its blood makes antibodies to the BSA. Giaever presents a drawing to show that it takes two weeks for the rabbit to manufacture the antibody. "I drew this rabbit—I have to tell you it's a rabbit, otherwise you might think it was a kangaroo. It would be all right if it was a kangaroo. All vertebrates and only vertebrates make antibodies. That's why there's no way chestnuts can cure themselves

from blight—likewise the elms from the Dutch elm blight." Rabbit serum containing antibodies to BSA had to be "supplied by a drug company. GE employs 350,000 people and not a single rabbit."

When rabbit serum containing the BSA antibody was dripped into the tank, another layer grew on the BSA. The extra layer was 50 angstroms thick. "It fits the size of the antibody," says Giaever. It took about an hour for the second layer to form, because the speed is limited by the speed with which the antibody diffuses through the solution. "We are seeing a chemical reaction as it happens because of the diffusion limitation," he says. The way the extra layer forms can be used to tell the concentration of antibody in the serum. In the specific case it was one hundred-thousandth of a gram per cubic centimeter.

"I thought it was a practical way to do clinical immunology," says Giaever. Since each antibody sticks only to its own antigen and vice versa, starting with a known antibody one can test serum for its antigen; starting with a known antigen one can test for its antibody.

The method is quicker than the usual clinical method, which consists of setting up an array of test tubes with varying quantities of antibodies in them and trying to precipitate out an antibody-antigen combination when the test samples are added. The slide-dipping method is also very sensitive to minute quantities of antibody or antigen. It is now about as good as the most sensitive alternative method, radioimmunoassay, measuring concentrations as low as a billionth of a gram per cubic centimeter.

"I showed it to some MD's and they were impressed," says Giaever, "but they thought it too complicated. I think everything is too complicated for MD's." How to make it simpler? Giaever remembered a property of indium metal.

When indium is deposited on glass it goes on in globules about 1,000 angstroms across. If an indium slide is dipped in the protein, the protein coats the globules. But this coating is visible to the naked eye since it changes the way the slide scatters light and so makes the slide look darker. Thus one doesn't need the complicated optical equipment of the ellipsometer. "The reason [for the darkening] is buried someplace in Maxwell's equations," Giaever explains. The double layer of antigen and antibody will look darker still.

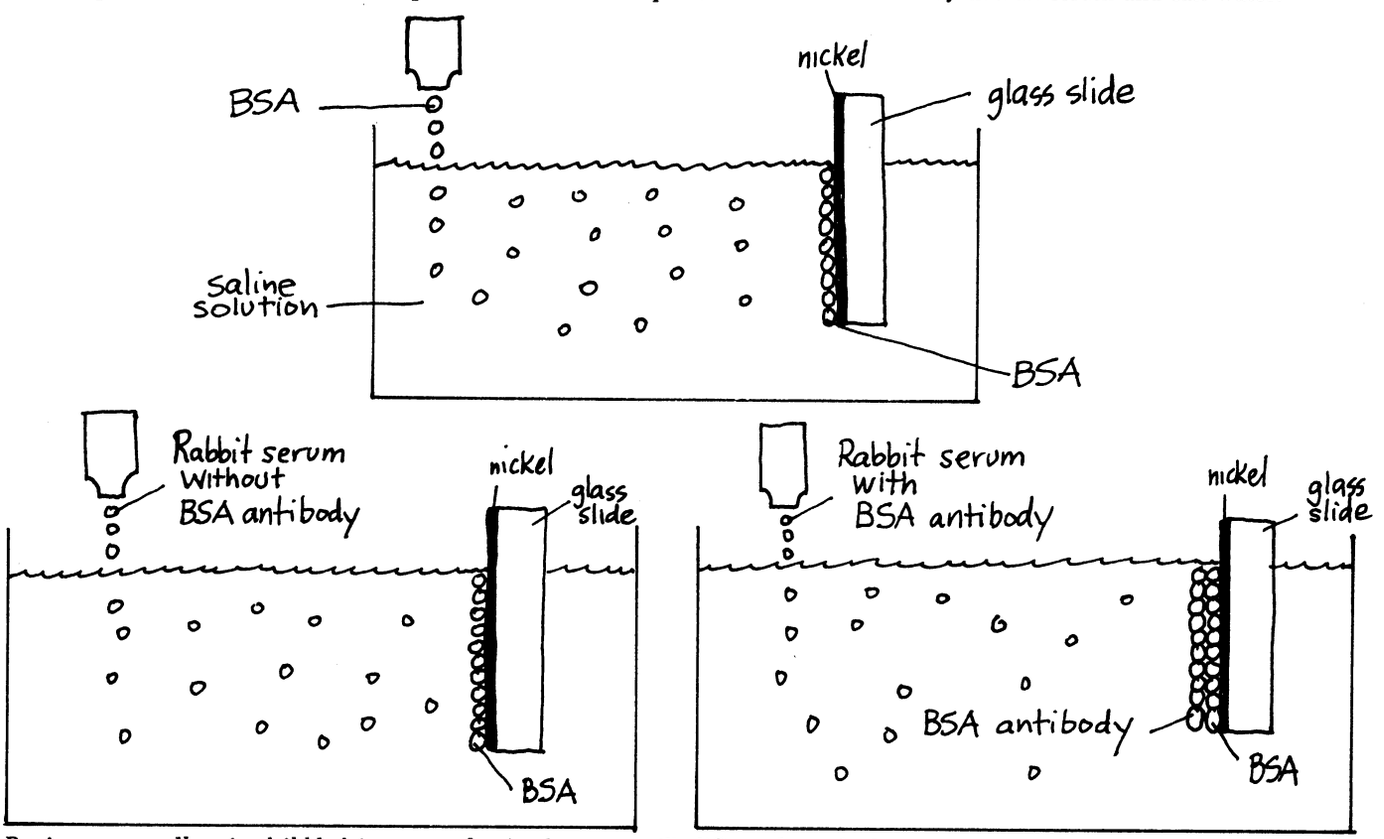
You can write on the slide with BSA. Dip the slide into rabbit serum without BSA antibody, and the letters disappear because proteins from the serum fill in around them. Then dip the slide into rabbit serum with BSA antibody. The letters reappear because the antibody sticks only to the BSA and not to the other proteins.

"This is something the doctor can understand," says Giaever. "They can write on the slide what ails you, and they're gonna read it out. They can even put the bill on the slide."

At about this time Giaever found out that he was doing the experiments in an unorthodox way. Chemists told him that when they worked with proteins in solution they used a phosphate buffer to keep the acid-alkalinity balance fixed. Giaever tried it. "Protein sticks to nothing in phosphate," he finds. He says he got an idea for a GE dishwasher detergent from this. But he's glad he didn't start his experiments in the orthodox way. "If I had known you use a buffer when I started, I would be talking today about electron tunneling."

There is already a quasiclinical use of the method, though Giaever warns it is still controversial. It concerns testing the blood of people who have gastrointestinal cancer to see how well treatment is progressing. The patients have in their blood minute quantities of a substance called carcinoembryonic antigen (CEA). Assaying the amount can tell whether they still have cancer or whether treatment is succeeding. Giaever is collaborating with physicians in Boston on tests of the blood of such subjects.

"I hope the indium slides may find clinical use," says Giaever. The method is simple, and the indium is unaffected by human serum and salt water. □



Bovine serum albumin dribbled into a tank of salt water will diffuse through the water and stick to a metal film (top). No proteins from rabbit serum will stick to the deposited BSA (left) unless the serum has the antibody to BSA (right).