Michael Heidelberger and the demystification of antibodies

Having defined the protein nature of antibodies under the tutelage of Oswald Avery, Michael Heidelberger was the first to apply mathematics to the reaction of antibodies and their antigens (the “precipitin reaction”). Heidelberger’s calculations launched decades of research that helped reveal the specificity, function, and origin of antibodies.

Heidelberger and Avery’s early experiments hinged on a simple element: nitrogen. Based on its presence or absence, the duo showed that the antibody-reactive substances on pneumococcal bacterial capsules were nitrogen-free carbohydrates, and that the reacting antibodies were nitrogen-containing proteins (see “How Heidelberger and Avery sweetened immunology” JEM 202:1306).

The discovery that antibodies are proteins was a notable achievement, particularly at a time when the nature of antibodies—substances known only to reside in proteinaceous serum—remained a mystery. But many contemporary immunologists consider the quantitation of the precipitin reaction an equally important accomplishment.

Obeying the law of mass action
Heidelberger and his postdoctoral fellow Forrest Kendall—who had been rendered one handed (but, by all accounts, no less dextrous) by a farm threshing machine—took advantage of purified bacterial polysaccharides (isolated during their earlier studies) for their quantitative experiments. They incubated varying proportions of purified antigen and antibody and determined the nitrogen content (i.e., antibody content) of the resulting precipitate. The data derived from these assays showed that the precipitin reaction could be expressed based on simple equations derived from the laws of mass action and that antibodies and antigens were multivalent. When they repeated the experiment with whole serum, their calculations held up, thus assuring them that their equations were not exclusively applicable to purified solutions (1,2).

An eye for color
“This was all very well for antibodies to polysaccharides,” noted Heidelberger in a 1979 article, “but what about those elicited by the vast numbers of protein antigens?” (3). As 15N had not yet been discovered, Heidelberger and Kendall instead used a colorful trick to distinguish antigen-derived nitrogen from antibody-derived nitrogen. They attached a purple dye to a model antigen—hen egg albumin—against which they raised specific antibodies in rabbits (4). The purple antigen, when incubated with specific antibody, created a colored precipitate. The amount of antigen-derived nitrogen in the redissolved precipitate could then be determined by comparing its color (by eye, as colorimeters did not yet exist) with solutions containing known concentrations of the dye; the remainder of the total nitrogen content was attributed to the antibody (5).

Multitalented proteins
Heidelberger later took on his first graduate student, Elvin Kabat. Together, they helped settle a longstanding debate concerning whether serum “precipitins” and “agglutinins” (antibodies that agglutinate bacteria) were the same or different. At the time, many people thought that these distinct functional properties of antibacterial antiserum could be ascribed to separate entities. Returning to the pneumococcal bacteria, the duo showed that precipitins and agglutinins were present in identical amounts in antipneumococcal serum. Reduction of one activity—by adsorbing the serum with a bacterial or polysaccharide solution—resulted in an equivalent reduction in the other activity, suggesting that the two functions were properties of the same antibody molecules (6). Kabat went on to show that antibodies in serum came in two sizes—large and small (now known as IgM and IgG)—based on their mass and sedimentation rate (7).

“Heidelberger’s work,” said former colleague Herman Eisner (Massachusetts Institute of Technology) in a 2001 article, “changed the concept of the antibody from an essentially ill-defined set of serum activities to a protein molecule, measurable in conventional chemical units...whose recognition of antigens could be analyzed in molecular terms” (8). Heidelberger, who was also a talented musician and linguist, worked in the lab until his death at age 103. At his 100th birthday party, he was reportedly asked how many papers he had published in his lifetime. “Three hundred and four,” he answered, adding slyly, “So far” (8).

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