THE CHEMISTS' WAR ON DISEASE

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For nearly a century and a half scientists have been studying ways of immunizing people against various diseases by artificial methods. Working mostly by trial and error they have devised reasonably safe vaccines and antisera for many diseases with a resulting spectacular decrease in mortality. The usual proceedure for vaccination is to inject the patient with the diseases-producing organisms which have first been killed or otherwise rendered harmless. Sometimes only bacterial products are injected. After vaccination the patient produces in his blood stream substances called antibodies which result in immunity. Serum from an immune person or animal (known as anti-serum) can be used to protect susceptible persons from the disease, e.g., diphtheria antiserum, or antitoxin, from the blood of horses previously injected with diphtheria toxin is in common use. Many puzzling problems still face these workers. In the case of many diseases the resulting immunity is only temporary, whereas for other diseases it lasts for many years. For many diseases there seem to be no effective vaccines. Often the most effective vaccines have a disagreeable effect on the patient.

In spite of the vast amount of research that has gone into developing practical vaccines and antisera, very little has been learned about the really fundamental principles of immunology, although it is apparent that such a knowledge would be of practical value in solving the problems just mentioned as well as being of purely scientific interest. Recently there has been a growing interest in making a direct attack on the problems of immunology in the chemistry department here at the Institute. Already there is a staff of several experienced immunologists and graduate students studying the problem from many angles.

Several investigators under the leadership of Professor Linus Pauling are attempting to discover the exact chemical composition and structure of antibodies, their methods of formation, and the details of the reactions by which they render invading organisms harmless. Such an application of ordinary physico-chemical principles to the processes occuring in living matter is in itself quite an advanced step. Chemists have traditionally confined their studies of reaction kinetics, molecular structure, etc., to relatively simple substances. It has generally been supposed that the reactions in living matter follow the same chemical principles which apply to reactions occuring in a test tube, but a detailed application of these principles to biological phenomena has generally proved impossibly difficult due to the complexity of living matter. Thus some of the simplest plant cells, organisms which have no apparent digestive system, can synthesize simple inorganic materials into organic compounds so complicated we have not yet succeeded in determining even their exact composition, let alone the processes by which they are formed.

Some of the members of the staff are trying to prepare antiboides synthetically in test tubes. It is their ultimate hope that in some cases we can produce antiserum synthetically which is superior to the natural product. Others of the group are studying the conditions under which an animal will produce natural antibodies to a given substance, with an eye to understanding why vaccines to some diseases prove ineffective or of only temporary protective power.

It is interesting to trace the steps by which immunology has evolved from Middle Age superstition to a modern branch of biology and chemistry. It was recognized many centuries ago that an attack of many infectious diseases conferred upon the patient immunity against further attack by that particular disease. Indeed there are records which show that attempts were made in ancient China and India to inoculate healthy individuals with the pus from smallpox pustules in the hope of producing a mild case of the disease which would result in continued immunity. These reports encouraged Jenner to devise a milder smallpox vaccination in 1798. At that time the bacterial cause of disease was still not recognized. However this imaginative and keenly observant English physician discovered that he could protect patients against smallpox by infecting them with mild cowpox.

People knew so little about disease then that practically no further progress in immunology was made until the work of Pasteur and his contemporaries nearly a century later. Pasteur not only extended Koch's proof of the bacterial cause of the

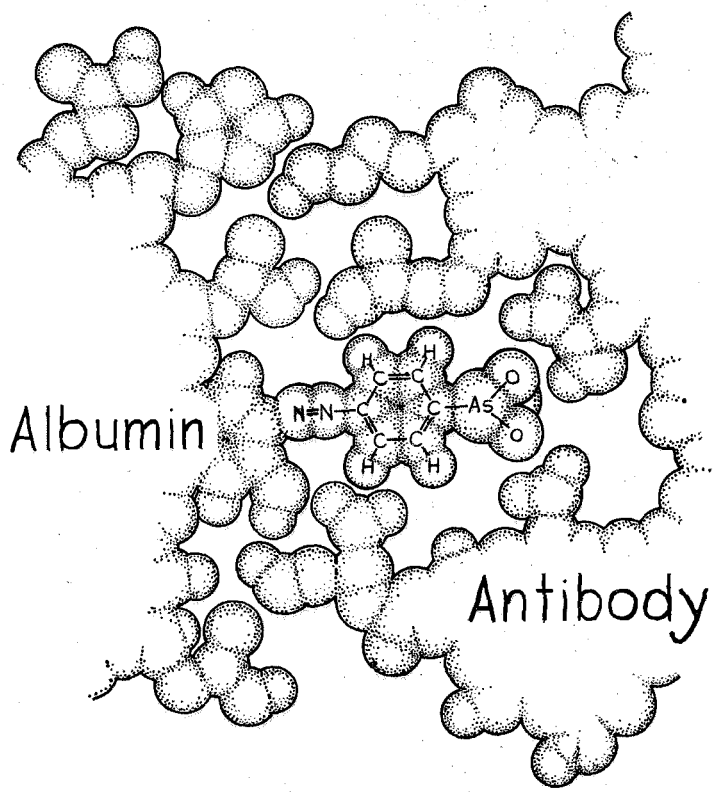

Fig. 1. A diagrammatic representation of a possible configuration for the antibody complementary to the antigen, arsanilic acid coupled to albumin. Weak bonds may form where the atoms (represented as spheres) of the antibody are near atoms of the antigen. About 35,000,000 times natural size.

disease anthrax in cattle to include many other diseases, but also discovered methods of treating virulent bacteria to render them harmless without destroying their effectiveness as vaccines. Until very recently further work in immunology has centered around isolating the organisms causing some disease and then finding a method of rendering them safe to use in vaccines.

The more imaginative investigators began studying immunology as a science in itself soon after Pasteur's time and discovered toward the beginning of the present century that the blood serum of immune individuals contains substances which give unique reactions in test tubes without the presence of living matter, i.e., *in vitro*. The first of these substances discovered were called agglutinins because they cause agglutination or clumping of the bacteria concerned. Then antiserum was found to contain precipitans which would precipitate the soluble material in bacteria. Later opsonins, antitoxins, and lysins with their own special effects were found. We now believe all these substances to be of essentially the same type and use the term antibody to designate the substance an animal produces in its serum when some foreign substance called an antigen, such as bacterial cells or a protein, is introduced. Furthermore, it is assumed that the process by which an individual fights off invading bacteria is essentially the same as the immunochemical reactions observed *in vitro*.

The next advance was quite recent work which shows that in many respects antibodies are not "bodies" comparable to cells at all, but are special proteins almost indistinguishable from serum globulin, one of the proteins normally present in blood.

The remarkable characteristic of antibodies is their high specificity. For example if hen egg albumin is the antigen injected into a rabbit, the resulting antibody in the rabbit's serum will combine best with hen egg albumin, to a lesser extent with related albumins, and not at all with serum albumin.

If either antigen or antibody is in large excess when the two are mixed they do not form a precipitate. This calls to mind similar observations in inorganic chemistry. For example, if silver ions and cyanide ions are mixed in sufficient concentration and in suitable proportions a precipitate forms. If the cyanide is in excess a complex forms and no precipitate appears. This type of reaction is not comomnly found among organic substances however.

Very few theories of the nature and formation of antibodies have been postulated. The first was Ehrlich's classical theory that antibodies are a normal component of the cell wall which aid in metabolism and which are released into the blood stream when an antigen is injected. Ehrlich did not present a detailed picture of antibodies and only intimated in a general way that thier specific reaction was due to their having a structure complementary to that of the antigen. Landsteiner and others experimented with hundreds of different artificial antigens and found that animals can produce antibodies highly specific for an infinite variety of antigens never found in nature. This demonstrated that antibodies could not be present in the animal before the antigen is introduced.

Two years ago Dr. Pauling proposed a theory of antibody structure and formation based on modern principles of structural chemistry, a field in which he is a recognized authority. The theory of the nature of the linkage between antibody and antigen when the two combine to form a precipitate had to fulfill certain unusual requirements. First, the bonding does not involve particularly active chemical groupings on the reactants as there are none available. Furthermore, the bonding is reversible and the antibody and the antigen can in some cases be separated from the precipitate in their original form. In these two respects the bonds are like certain highly nonspecific and relatively weak bonds involved in adsorption, crystallization, etc. These bear the names polar bonds, hydrogen bonds, and Van der Waals forces. Landsteiner has shown that even antibodies to optically active isomers, compounds identical except that one is a mirror image of the other, are so specific that the cross reaction of one with the opposite isomer is relatively weak. The unique feature of Pauling's theory is a detailed explanation of this high specificity in terms of ordinary chemical bonds. The antibody to a particular antigen is postulated as having a structure complementary to the antigen in two respects. First, to a certain extent its shape is such that it roughly fits the contour of the antigen. Furthermore, several sites on the antibody where hydrogen bonds, etc., can form are opposite corresponding sites on the antigen. Fig. 1 shows in very diagrammatic form a possible structure of a portion of an antibody complementary to arsanilic acid coupled to egg albumin. The antibody must have at least two such complementary regions in order to form the framework of molecules that makes up the final preciptates as shown in Fig. 2. Soluble

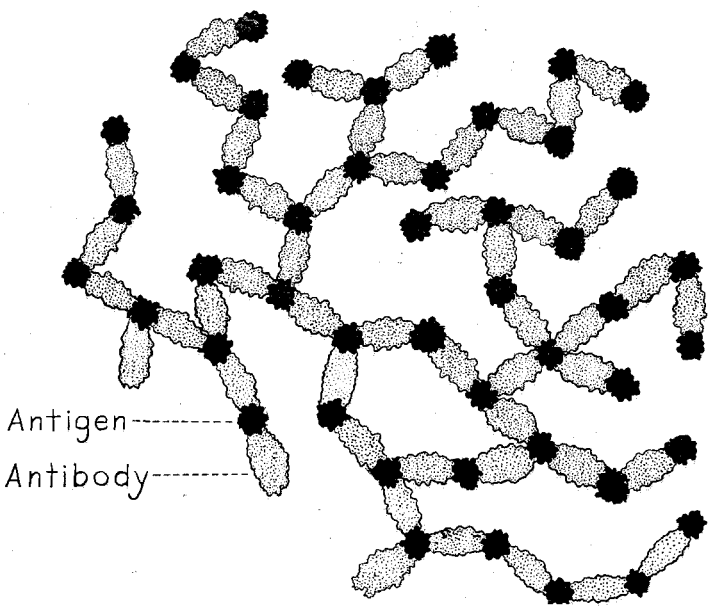

Fig. 2. The precpiitate of antigen and antibody consisting of a framework of antigen molecules joined by antibody molecules having two complementary regions. About 1,000,000 times natural size. (After Pauling)

complexes are formed when all the complementary regions on all the antibody or all the antigen molecules (depending on which material is in excess) are occupied before the framework forms. Agglutination of cells is essentially the same except that we are dealing with much larger particles, Fig. 3.

Several interesting phenomena of immunochemistry are explained by this theory. Fig. 4 illustrates typical examples of some of these. Consider the antigen *p*-nitro-aniline coupled to egg albumin. The complementary antibody obtained from rabbits injected with this compound will react with *p*-nitro-aniline coupled to egg albumin or any other protein or to benzene ring. Likewise it will react to a lesser degree with *p*-toluidine coupled to any protein because this molecule is structurally very much like *p*-nitro-aniline. Furthermore it will react with egg albumin alone. However this reaction with egg albumin will not occur if any large molecule which gets in the way is coupled to it. Also uncoupled *p*-nitro-aniline, if present in the solution in sufficient concentration, will inhibit any of these reactions by occupying the complementary sites of the antibody.

Experiments of the type just mentioned may prove useful in structural chemistry for comparing the structure of a known with an unknown compound. Thus if the antibody to the unknown compound also reacts with the known, we can assume that they both contain similar chemical groupings. This method is particularly adaptable to complicated compounds of biological origin which cannot be studied by election diffraction, x-rays, or spectroscopy due to their complexity. As our knowledge of immuno-reactions increases, especially in regard to the quantitative studies now in progress, and as we learn more about preparing satisfactory antigens of known structure, we believe that immunochemical reactions will be of primary importance to the structural chemist. Even many years before this, biologists have frequently used immunological reactions to show the similarity in structure of proteins from various species of animals and thus show that the species are probably related.

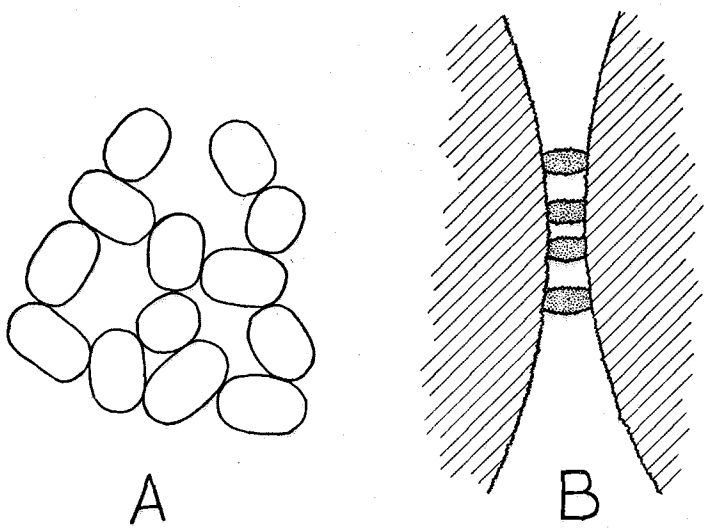

Fig. 3. (A) Diagram representing agglutinated cells. About 10,000 times natural size. (B) Diagram of the region of contact of two cells, showing four antibody molecules joining the cells. (after Pauling)

Pauling's theory also goes into a possible process for the formation of antibodies based on current theories of protein structure. Normal globulin, the protein most closely related to antibodies in most animals, is thought to be a long polypeptide chain of about 1,000 or 1,500 amino acids. This chain is folded back and forth on itself to make a compact, slightly elliptical molecule, the folds of the chain being held together by the weak bonds mentioned above. There are about 12,000

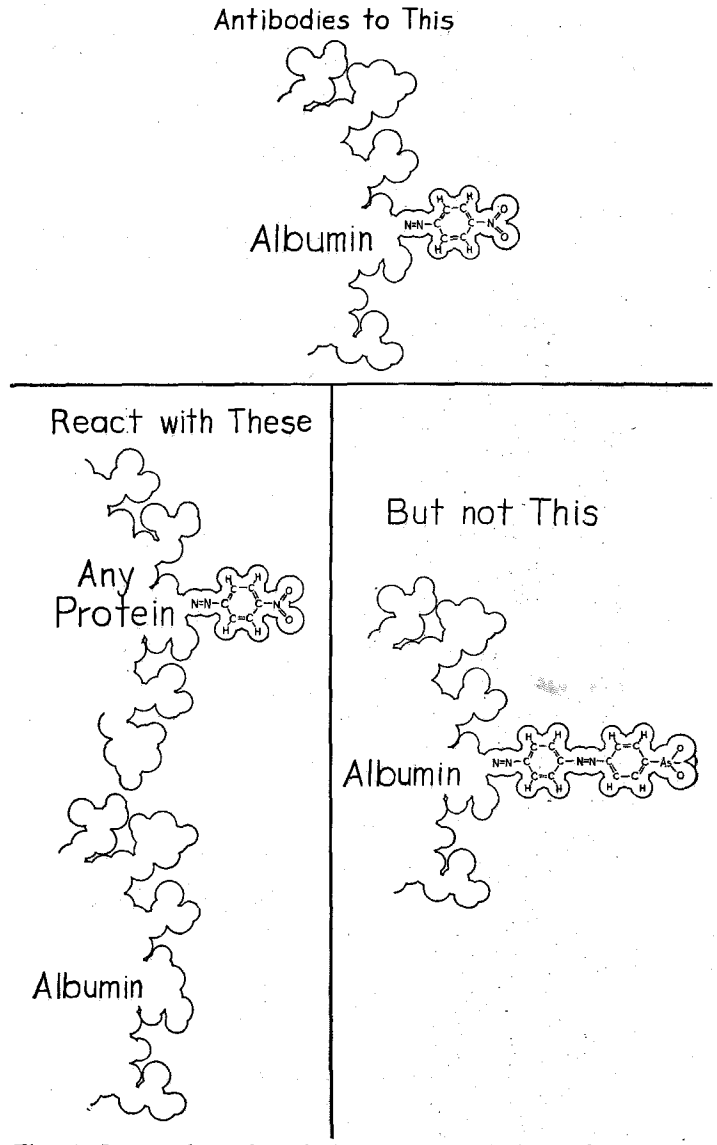

Fig. 4. Illustration of typical immunochemical reactions.

atoms of various elements in the entire molecule and the order of 400 on the surface of one end. Antibodies, according to the new theory, contain exactly the same polypeptide chain as normal globulin and differ only in the pattern of the folding. If an antigen particle is present during the folding process it pulls the chain around it by forming bonds to suitable groupings of the chain with the result that the globulin folds into the specialized complementary configuration of an antibody.

It occurred to Dr. Pauling that it might be possible to make antibodies synthetically if the process by which they are formed naturally is indeed the one mentioned above. Experiments to this end have been encouraging almost from the outset. All the methods tried have in common some means of unfolding the normal globulin chain and refolding it in the presence of the antigen. Experiments on denaturation have already shown that this can be accomplished by mild heating, treatment with alkali, or by spreading the protein on a liquid surface. All these methods have been tried for producing synthetic antibodies. The most successful procedure developed so far is to heat together a solution of normal globulin and the desired antigen, holding the temperature at 57° C. for several days.

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In this way antibodies to several simple antigens and, we believe, to various proteins like egg albumin have been prepared. They behave much like the natural antibodies in many respects. Since one of our goals is the preparation of synthetic antisera, Dr. Dan Campbell has started a series of experiments on synthetic antibodies to various bacteria. He has chosen to start on the pneumococci, which are relatively easy to work with and are at the same time very important in medicine. The synthetic antibody produced will be tested for its ability to actually protect mice against pneumonia in the same way that the natural pneumonia antiserum now in common use is tested before being distributed for use.

The possibility of making synthetic antisera for treatment of disease is something to stir one's imagination, especially under the urgent needs of war-time conditions. Making them naturally has always been a slow and involved process. It may be that we can make them quickly and in large quantities in the vats of a chemical works. Also the synthetic antibodies should be relatively free from the foreign substances that occasionally cause serum sickness when the use of present day antisera is not carefully controlled. It is not unlikely that certain infectious diseases which cannot be handled by the classical methods will succumb to the synthetic treatment.

There has been some question as to whether the particular type of antibodies with which we have been working, namely precipitins, which precipitates antigens in solution, are the same as the agglutinins which cause bacteria and other cells to clump, tbus helping to render them harmless. Dr. David Pressman has performed some interesting experiments to add strength to evidence obtained in other laboratories that there is no essential difference between them. First he tested Pauling's postulate that an antibody or other molecule capable of combining with two bacterial cells could cause agglutination. Using diazotized benzidine which can link with two protein molecules, he simulated the agglutination of red blood cells. Then he caused the precipitin for egg albumin to agglutinate red cells simply by first coating the cells with albumin. Other workers have performed the converse experiment, showing that the antibody which agglutinates pneumococci will also precipitate the material which coats the organisms.

Reference was made in the introduction to a study of the kinectics of immunological reactions at The Institute. Ordinarily the first step in such a study would be to get pure materials and select a clean cut reaction that gives products of known and constant composition. That is not possible in immunology. Antiserum contains the normal components of blood as well as the antibodies of interest. Probably the antibody molecules to a particular antigen are quite inhomogeneous. Some have only one complementary combining region and others two or more. The resulting precipitate has a variable composition. In spite of these difficulties Dr. Pauling has derived rather complicated expressions relating the composition and amount of precipitate with the concentrations of antibody and antigen, the equilibrium constant for their combination, and the effects of inhibiting agents. Dr. Pressman and assistants have been testing these expressions in a series of experiments involving thousands of analyses. A great simplification was introduced when it was discovered that relatively simple organic dyes of known structure and high purity could be used for the antigen. Experiments of this type add greatly to our knowledge of the manner in which antibodies combine with various antigens.

A still more direct attack on the structure of antibodies is to be made this winter with the use of a new electrophoresis apparatus which I constructed last year. It is still a matter of conjecture as to how many places on an antibody can combine with the antigen molecules. In these new experiments the antibody will be allowed to form soluble complexes with very simple antigens. When an electric current is passed through the mixture the antibody molecules will separate into distinct layers. With the new apparatus it will be possible to measure the amount of antibody in each layer and also to test the uniformity of any particular layer. The material in each layer can be collected for further experiments.

It should be mentioned that these experiments on antibodies probably have a much broader significance than just their relationship to immunity. In all living matter there is a great variety of highly specific reactions. Enzyme action, the fertilization of eggs, certain steps in cell division, allergy, and the synthesis of proteins are but a few examples. It seems likely that the type of bonds involving complementary structure which have been discussed in this paper play a leading role in many of these life processes. In view of the excellent progress in immunochemistry made at the Institute last year and the increasing interest being shown, important results should appear during the next year.

Western War Minerals

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for all our needs are readily obtained as a by-product from nickel mining in Ontario.

The demand for radio-sending and detection devices of all types in this war has increased tremendously the need for quartz crystals, which are a vital part of many such units either as a frequency control, a crystal detector, or because of the piezoelectrical properties. Miscellaneous uses of strategic interest include range-finders, instruments measuring pressures or detonation in gun barrels or airplane engines, in depth sounding and direction finding apparatus, and for sundry precision instruments such as chronometers, seismographs, periscopes, gun-sights and polariscopes. These quartz crystals must be free from flaws of all types, twinning, impurities, intergrowths and fractures. Most of it occurs in igneous rocks or veins from which it only can be separated by explosives which fracture the quartz. Our supplies normally are obtained almost entirely from Brazil, where the quartz crystals are found in clays resulting from the long-continued weathering of igneous rocks. By this process the matrix around the quartz is decomposed and the quartz liberated.

Our imports increased from about 10 tons in 1936 to 63 tons in 1940. There are one or two deposits in California where optical quartz can be mined. Weathering conditions throughout the west however, have not favored the liberation of optical quartz from our existing deposits.