The antibody handshake

Wrestling recently with an unpleasant bout of influenza, your writer consoled himself with the thought that his immune system would soon dispatch the unwanted virus. And, much to his relief, it did. The welcome recovery depended on antibodies latching on to antigens of the virus, and new details of the way in which they do that have recently emerged from the CSIRO Division of Biotechnology.

One of the main weapons of the vertebrate immune system is the production of antibodies. These are protein molecules of many different types, each with a particular configuration allowing it to bind to a specific foreign compound that may enter the body on the surface of invading micro-organisms. The binding neutralises or inactivates the foreign substance, termed an antigen.

Many antigens are, like antibodies, protein molecules. However, they need not always be, for an antigen is, by definition, any substance that can elicit the production of antibody. But when the immune system deals with pathogenic invaders, rather than noxious substances as it occasionally does, then most antigens are proteins, and the coming together of antigen and antibody is a special type of protein–protein interaction.

Variable numbers (from a handful to thousands) of about 20 different types of amino acids make up all proteins. The amino acids string together to form chains called polypeptides and a protein may contain one or more of these. Antibody molecules contain four chains. Some proteins may have sugar molecules attached to them, in which case they are, strictly speaking, called glycoproteins.

Two of the most important external parts of the flu virus are protein antigens. They are neuraminidase and haemagglutinin (both glycoproteins), and scientists working on understanding the virus and — hopefully — providing a cure for the disease have studied these two components in great detail. After mapping the structure of the enzyme neuraminidase (see *Ecos* 38), Dr Peter Colman and his colleagues — Dr Peter Tulloch, Dr Jose Varghese, Dr Tony Baker, and Mr William Tulip — of the Division of Biotechnology's Melbourne laboratories, have now studied the binding of an antibody to it.

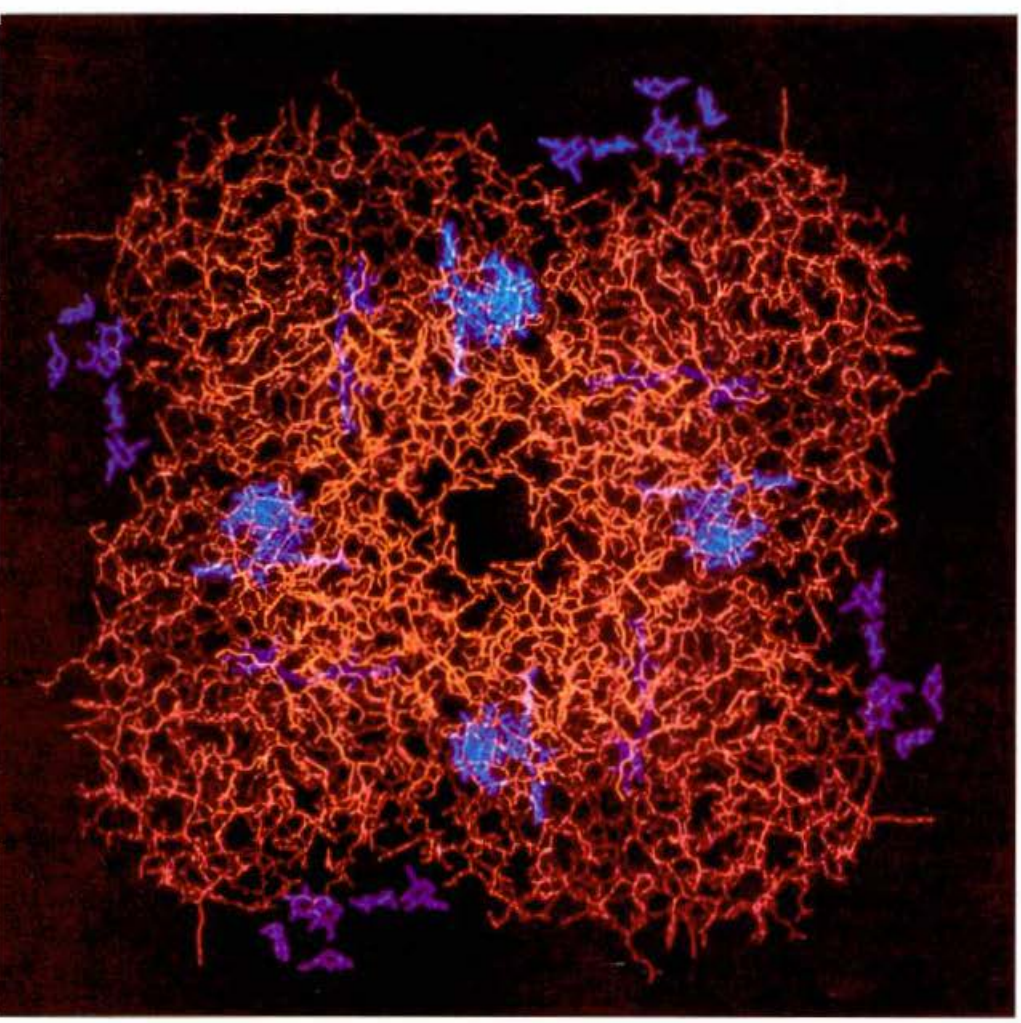
Starting with crystals of anti-neuraminidase antibodies already attached to neuraminidase itself, Dr Colman used the technique of X-ray diffraction to obtain a detailed three-dimensional picture of the 'embrace' of the two proteins. (This work is in collaboration with Dr Graeme Laver of the Australian National University, and Dr Robert Webster and Dr Gillian Air, in the United States of America.)

The 'Y' and how of antibodies

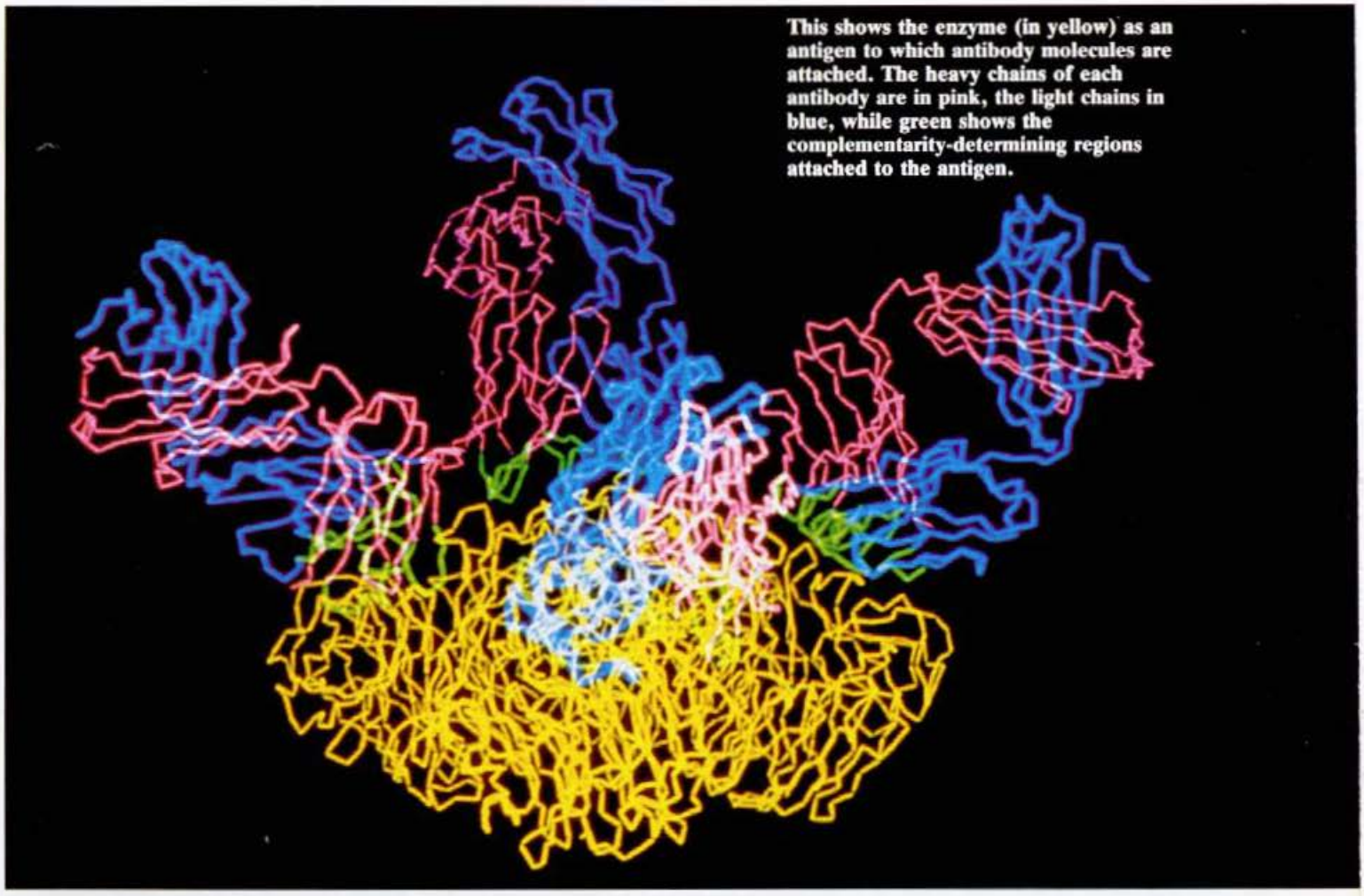
Immunologists know that antibodies are Y-shaped molecules, with each molecule being composed of four peptide chains — two light and two heavy. A complete light chain and part of a heavy chain make up an arm, and the two arms comprise the antigen-binding site.

...an important new insight into how the immune system works.

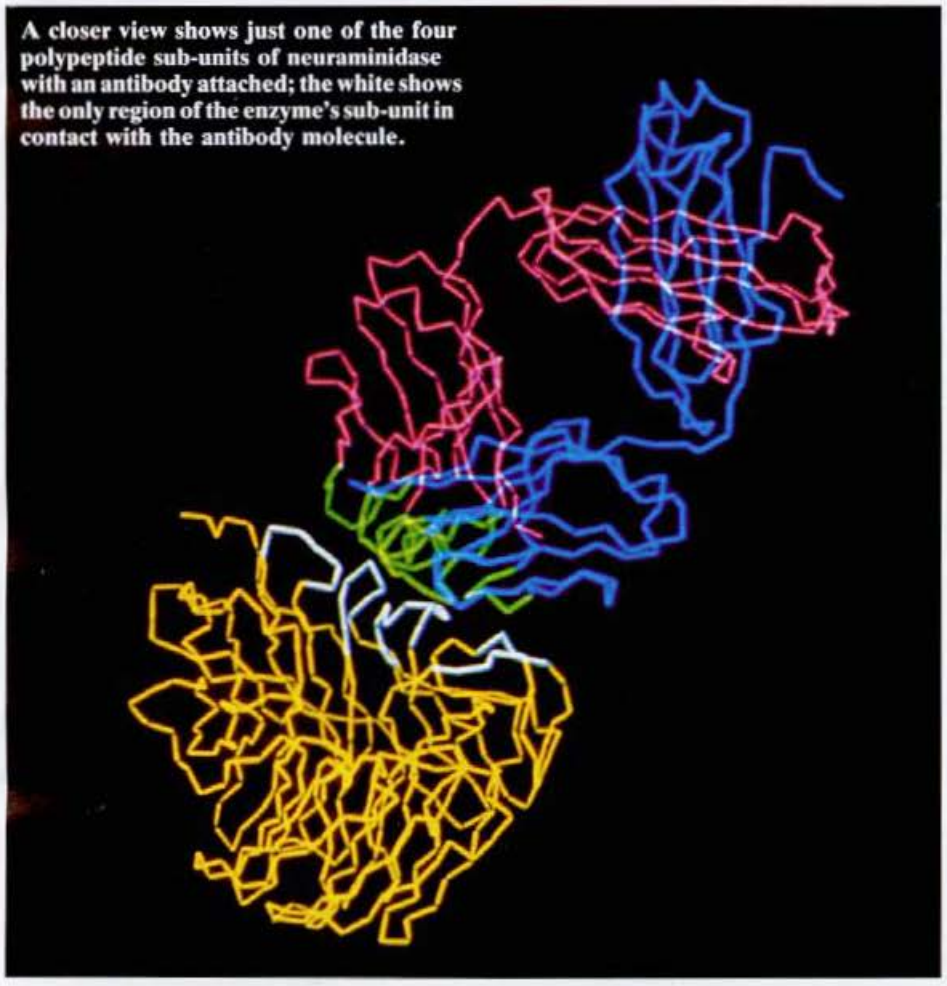
Each arm contains a variable zone, made up of a portion of the light and heavy chains. This includes a number of what prolix immunologists term 'complementarity-determining regions'. The sequence of amino acids in such a zone is different in each antibody, providing the variation in structure necessary to accommodate binding to the millions of different foreign chemical shapes — located mainly on invading organisms — that we are likely to come across in a lifetime.



A view of the the viral enzyme neuraminidase, showing all its atoms. You can see that it is divided into four parts. The sugar components of it are in blue.



This shows the enzyme (in yellow) as an antigen to which antibody molecules are attached. The heavy chains of each antibody are in pink, the light chains in blue, while green shows the complementarity-determining regions attached to the antigen.



A closer view shows just one of the four polypeptide sub-units of neuraminidase with an antibody attached; the white shows the only region of the enzyme's sub-unit in contact with the antibody molecule.

It is one of the most awe-inspiring facts in biology that our bodies have the potential to produce so many different types of antibody (estimated at about 10–100 million), specific to such a wide range of antigens, many of which we may never actually encounter. For example, we have the ability to make antibodies whose variable regions will bind specifically to protein antigens on sheep red blood cells, although in the normal course of events we are unlikely to be 'invaded' by sheep's blood.

This is sometimes explained by the idea of cross-reactivity. The sheep red-blood-cell antigens may resemble antigens found on bacteria, and those specific antibodies we carry were perhaps ready for an encounter with such bacteria. The possible close molecular resemblance of the sheep antigen to a similar bacterial antigen allows the specific antibodies to cross-react with it, although exactly what was happening on a molecular level had, until Dr Colman's findings, not been fully explained.

A similar phenomenon occurs with enzymes. They are also protein molecules, and their job is to catalyse the chemical reactions of life. Like antibodies they are specific to their substrates (that is, the molecules with which they react). How-

ever, some cross-reactivity can occur if two substrate molecules resemble each other sufficiently. Then, both will be able to fit into the 'active site' of the enzyme. Providing a false substrate to do this and hence inactivate the enzyme is the basis for the action of many drugs.

Substrate molecules are said to fit into an enzyme's active site like a key into a lock — a good metaphor, as it explains the specificity of the reaction between the two molecules as well as the importance of complementary shape. It is also valid because the active site is only part of the enzyme, just as only part of a lock holds the key.

But the lock and key model of enzyme action has recently been modified as evidence has accumulated to suggest that the interaction of an enzyme with its substrate is not nearly as rigid as the model implies. Instead, biochemists now speak of 'induced fit' where, after initial binding based on complementarity of shape and electric charge, the substrate and enzyme molecules may each distort slightly to clasp each other in a better fit.

The usual theory of the interaction between an antibody and its target antigen states that, like a lock and key, the binding region of the antibody is a specific shape, complementary to a portion of the antigen molecule. Previous work supported this idea by suggesting that the molecular configuration of the antibody's arms did not change when antigen was bound.

But, in a world first, Dr Colman and his colleagues have demonstrated that an antigen's structure can change when binding with an antibody and their work has suggested the possibility that the reverse may also be true. Using an electron microscope as well as X-ray analysis, the team obtained a detailed picture of the antigen–antibody complex.

Handshake

This showed that, as with enzyme–substrate interactions, the 'lock and key' model is not accurate. The team have suggested that the pairing of the variable regions of the light and heavy chains in the antibody's arms can change when the antigen binds. The scientists also noticed that part of the antigen was perturbed by its interaction. They believe that portions of the antibody's arms actually slide, moving the important complementarity-determining regions by about 0·3 of a nanometre. (One million nanometres equal one millimetre.)

The fact that part of the antigen is also deformed by the antibody means that the interaction should be viewed more as a handshake than a key in a lock. As the two molecules come into contact, so they seem to modify each other to achieve a better fit.

We know little about how antibodies neutralise viruses. The work of Dr Colman and his colleagues suggests that one possible mechanism may involve the capacity of the antibody to distort the antigen's original structure. Before they can say whether or not that is the case for the particular antibody they have used, the scientists need to investigate further. Also, of course, binding of the antibody may affect the enzyme in other ways, such as physically getting in the way of the substrate as it approaches the active site.

Implications

Immunologists will be looking at the CSIRO finding very closely. Whether these changes happen with all antibody–antigen interactions we don't yet know. Scientists will need to test this with a whole range of different antigens.

However, the work of Dr Colman and his colleagues provides an important new insight into how the immune system works. If antibodies can change to accommodate an antigen, this may help to explain how they can bind more than one antigenic type. Dr Colman has recently shown that the opposite situation, where two different antibodies bind to the same place on an antigen (neuraminidase again), can occur, although this too had not previously been considered likely.

Conformational change in antibodies possibly explains how the immune system can cope with an almost infinite array of antigens. Our ability to make 10–100 million different antibody types is by itself probably not sufficient to serve us in a world with so many subtly different chemicals on such a wide variety of different infectious organisms. However, one antibody binding a number of superficially dissimilar antigens gives the immune system greater flexibility.

Of course, this does not mean that a 'free-for-all' exists where anything goes between any antibody and any antigen. A certain amount of specificity must remain, otherwise flexible antibodies could attach to anything, including our own body components. (With certain auto-immune disease states they do in fact do this, for reasons presently not well understood. Dr Colman's discoveries may shed some light on this too.) But the idea of an absolute 'one-to-one' relationship of one type of molecule to one uniquely specific, complementary antibody must go. However, the immune system, despite intensive study over the last few decades, still keeps plenty more secrets!

Roger Beckmann

Towards a flu cure

The flu virus enzyme neuraminidase, itself a sugar-protein or glycoprotein, acts to split a modified sugar molecule called neuraminic acid (or sialic acid) off certain other complex compounds. Various fats and proteins may have sugar groups attached to them, in which case they are called, respectively, glycolipids and glycoproteins. Different versions of neuraminidase can catalyse the reaction on either of these.

The importance of neuraminidase to the virus — both in allowing it passage through mucus and enabling new virus particles to leave an infected cell — is well known (see the other box), and now the goal of the CSIRO scientists, with others from the Victorian College of Pharmacy, is to find out how to block the enzyme's function. Together, they are studying the structure and mode of function of neuraminidase to provide them eventually with enough knowledge to design an effective inhibitor compound that would attach to the enzyme and prevent it catalysing its reaction.

One difficulty is that neuraminidase is fairly common. Our own bodies make their version of it, and so do many bacteria. Dr Elizabeth Cartwright, a member of Dr Colman's team, is trying to isolate, purify, and crystallise a mammalian neuraminidase in order to determine its structure, and see how it differs from the viral form. Already she has found that there are many different types of neuraminidase even within the different tissues of one body, and these forms may have different properties.

Scientists already know of lots of possible inhibitors for this enzyme, such as modified substrates and various metal ions. The trick will be to find one that remains attached to the viral enzyme *in vivo*, and that does not adversely affect the various types of host neuraminidase. Such a drug would not necessarily protect against the flu in the way that a vaccination might, but could quickly clear up the disease once you already have it.

More about the topic

Three dimensional structure of a complex of antibody with influenza virus neuraminidase. P.M. Colman, W.G. Laver, J.N. Varghese, A.T. Baker, P.A. Tulloch, G.M. Air, and R.G. Webster. *Nature*, 1987, **326**, 358–62.

Portrait of a disease

In 1918/19, just as World War I was drawing to a close, a scourge of equal devastation (rarely reported in the history books) quietly occurred. It was a plague of influenza that swept across the world killing about 20 million people (more than died in the fighting on all sides during the war).

In fact, many of the deaths were brought about by pneumonia and other infections caused by bacteria, but it was the severe influenza that, by weakening the body — especially in the very young and old — allowed the bacteria to take hold.

Not all strains of influenza are as severe as the one that caused that pandemic. New strains arise every so often by a process of genetic recombination between different virus types (see *Ecos* 31 for the details). This accounts for the difficulties your immune system, or a vaccination, has in affording you true protection. Antibodies to one strain protect you only as long as that type is in circulation. A new strain is antigenically different.

Virus is released into the air when infected people talk, cough, or sneeze. If you breathe in virus particles, they will attach to some of your cells by means of the haemagglutinin spikes that stick to specific glycoprotein receptors on the cell membrane — unless you have a high titre of antibodies to haemagglutinin in your mucus. (Glycoproteins are proteins with modified sugars attached.)

Not all your cell types have these receptors, but those lining the respiratory system do, and that is where the virus strikes. It is rare in a normal person for the virus to spread out into the bloodstream and the rest of the body.

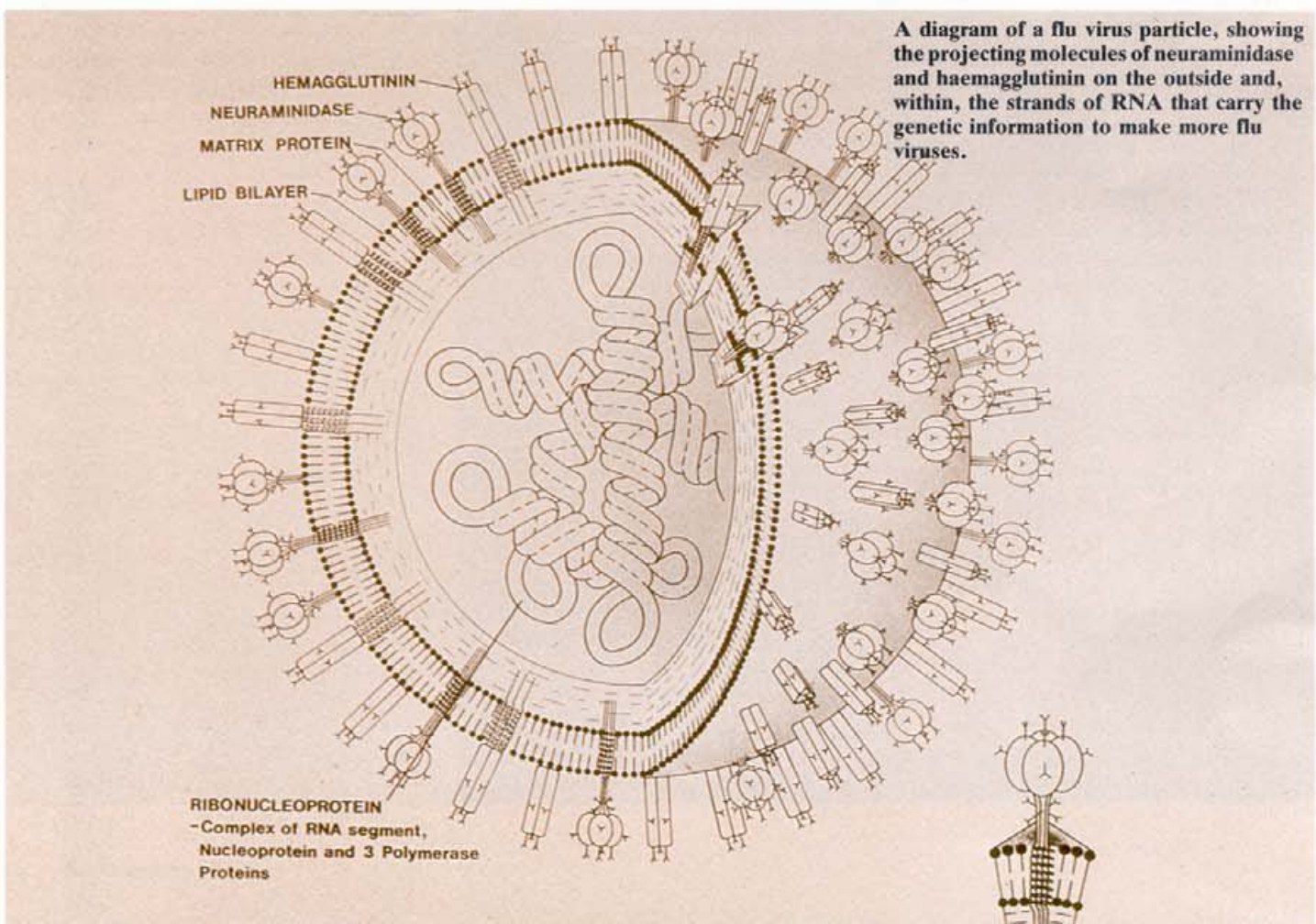
Unlike antibodies to haemagglutinin, those against neuraminidase will not prevent the virus entering your cells. However, neuraminidase is important in allowing new virus particles to leave cells. A few hours after a cell in, say, the lining of your nose is infected by a single virus particle, thousands of new particles will bud out from the cell membrane, leaving the devastated cell to die.

The enzyme neuraminidase goes into action now to cut the new virus particles free from the cell membrane. Antibodies to neuraminidase are thus important in limiting the spread of the virus in your body, and so speeding recovery — but they will not act to protect you from infection in the first place.

Mucus contains glycoproteins, and neuraminidase further aids the virus by catalysing the removal of sialic acid from these. Now, usually, the layer of mucus that coats your respiratory epithelia acts as a very efficient barrier to the entry of many bacteria and viruses. For example, its glycoproteins — similar to those on cell membranes — could stick to flu virus particles and hold them fast were it not for the action of the virus' neuraminidase that, by partly digesting the glycoproteins, helps the virus get through to reach the cells. (The enzyme's work renders the mucus thin and watery — something you may notice as you reach for yet another handkerchief during the early stage of the disease.)

The cells lining the upper respiratory tract are killed by the infection, and the systemic effects of the disease — such as fever and muscular aches — are probably brought about by products released during the break-down of these cells and also by white blood cells involved in removing the associated debris.

But although the infection is self-limiting — once you have formed antibodies to neuraminidase and infected cells have died — the virus has a final card to play. Infection appears to cause a degree of inhibition of phagocytosis. This is the process whereby some white blood cells engulf and digest bacteria. If phagocytosis is less effective, then bacteria can spread more easily within the body, and this accounts for the worrying associated infections — such as bacterial pneumonia — that can occur with influenza.



A diagram of a flu virus particle, showing the projecting molecules of neuraminidase and haemagglutinin on the outside and, within, the strands of RNA that carry the genetic information to make more flu viruses.