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The Structure of Antibodies

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# The Structure of Antibodies

The basic pattern of the principal class of molecules that neutralize antigens (foreign substances in the body) is four cross-linked chains. This pattern is modified so that antibodies can fit different antigens

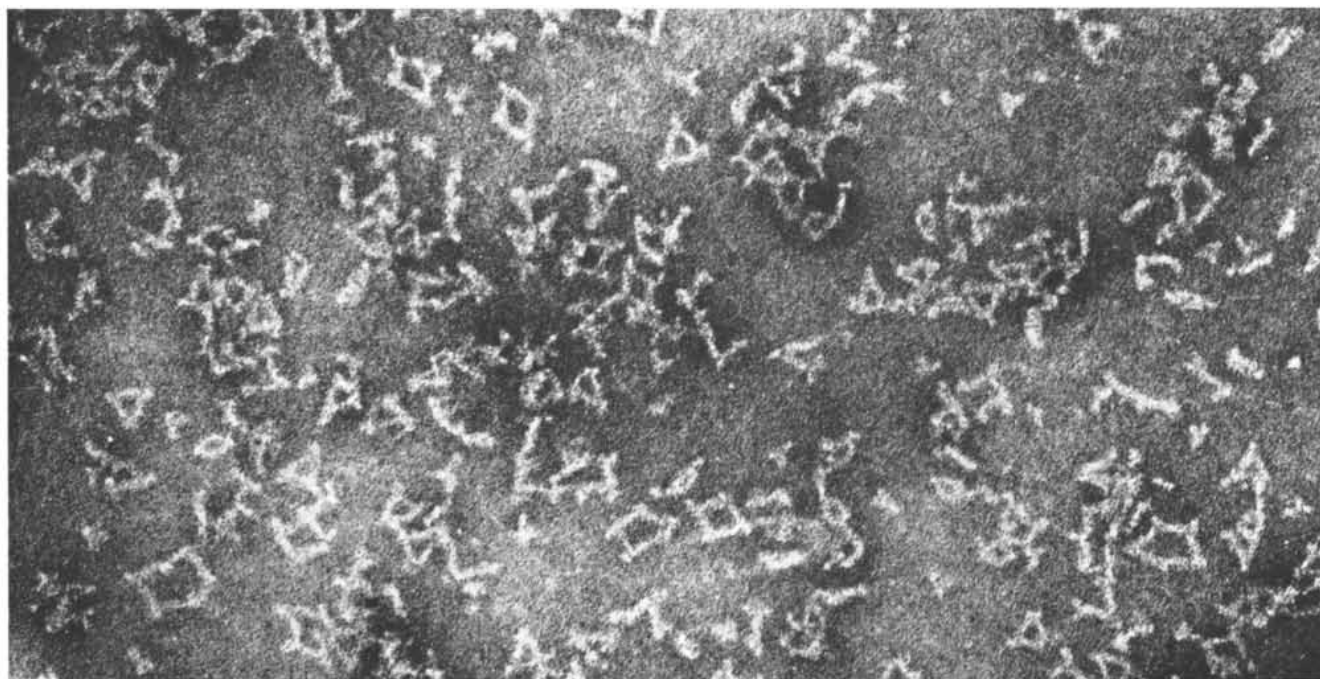
by R. R. Porter

It has been known for millennia that a person who survives a disease such as plague or smallpox is usually able to resist a second infection. Indeed, such immune people were often the only ones available to nurse the sick during severe epidemics. A general understanding of immunity had to await the discovery that microorganisms are the causative agents of infectious disease. Then progress was rapid. A key step was taken in 1890 by Emil Von Behring and Shibasaburo Kitasato, working in the Institute of Robert Koch in Berlin. They showed that an animal could be made immune to tetanus by an injection of the blood serum obtained from an animal that had survived the disease and had

developed immunity to it. Serum is the clear fluid that is left behind when a blood clot forms; it contains most of the blood proteins. Thus immunity to tetanus is a function of a substance or substances in the blood. These substances were named antibodies.

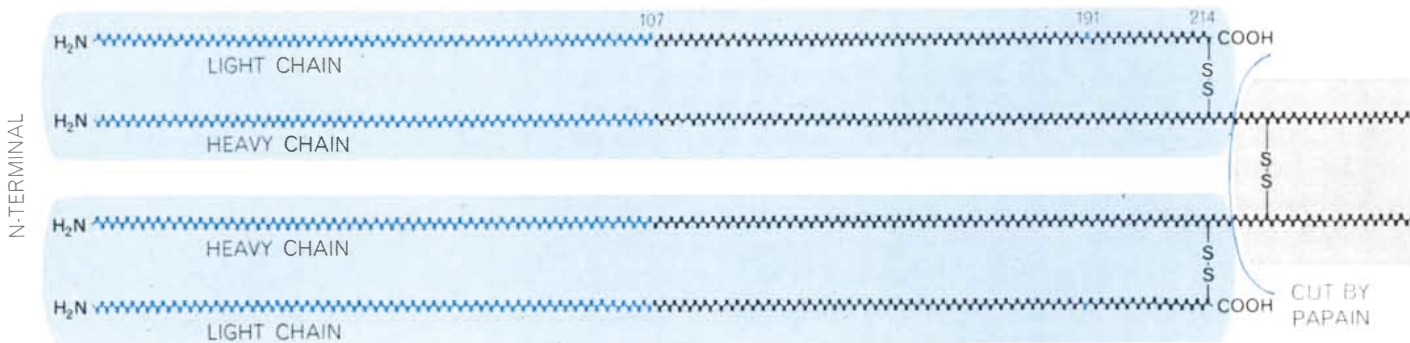
Antibodies are produced by all vertebrates as a defense against invasion by certain foreign substances, known collectively as antigens. The most effective antigens are large molecules such as proteins or polysaccharides (and of course the microorganisms that contain these molecules). The demonstration of the appearance of antibodies in the blood is most dramatic if the antigen is a lethal toxin or a pathogenic microorganism:

the immune animals live and the non-immune die when injected with the antigen. Innocuous substances such as egg-white protein or the polysaccharide coat of bacteria, however, are equally effective as antigens. The antibodies formed against them can be detected by their ability to combine with antigen. This can be shown in many ways. Perhaps the simplest demonstration is provided by the precipitate that appears in a test tube when a soluble antigen combines with antibody contained in a sample of serum. The most remarkable aspect of this phenomenon is the specificity of the antibody for the antigen injected. That is, the antibody formed will combine only with the antigen injected

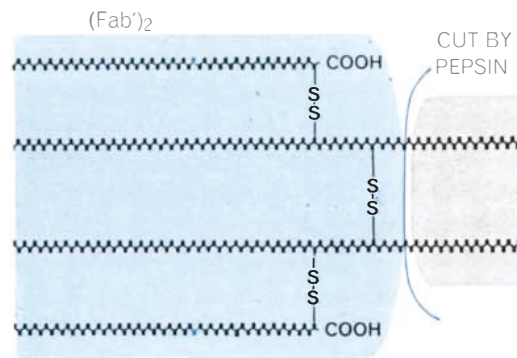


ANTIBODIES BOUND TO ANTIGENS are depicted in this electron micrograph made by Michael Green and Robin Valentine of the National Institute for Medical Research in London. The antigen

itself is too small to be visible, but it evidently acts as the coupling agent that binds antibody molecules together to form the various multisided structures. The magnification is about 275,000 diameters.



**IMMUNOGLOBULIN GAMMA**, the chief class of antibody, is a protein molecule consisting of four polypeptide chains held together by disulfide (S—S) bonds. The two light chains are identical, as are the two heavy chains. Depending on the source, the light chains contain from about 210 to 230 amino acid units; the heavy chains vary from about 420 to 440 units. Thus the lengths, the spacing between disulfide bonds and enzyme cleavage points shown here are approximate. The enzyme papain splits the molecule into three fragments (*above*): a fragment that forms crystals (Fc) and two fragments (Fab) that do not crystallize but contain the antigen binding sites. Approximately half of each Fab fragment (*color*) is variable in amino acid composition. Site 191 is genetically variable. When immunoglobulin gamma is split by the enzyme pepsin (*right*), the Fab fragments remain bonded together ( $(Fab')_2$ ) because the cleavage occurs on the other side of the central disulfide bond.



or with other substances whose structure is closely related.

Numerous different antibodies can be formed. Although an individual animal may respond poorly, or perhaps not at all, to a particular antigen, there is no known limit to the number of specific antibodies that one species, for example the rabbit, can synthesize. Conceptually there is a great difference between the capability of one species to synthesize a very large but limited number of antibodies and the capacity to synthesize an infinite number, but an experimental decision as to which is correct is not possible at present.

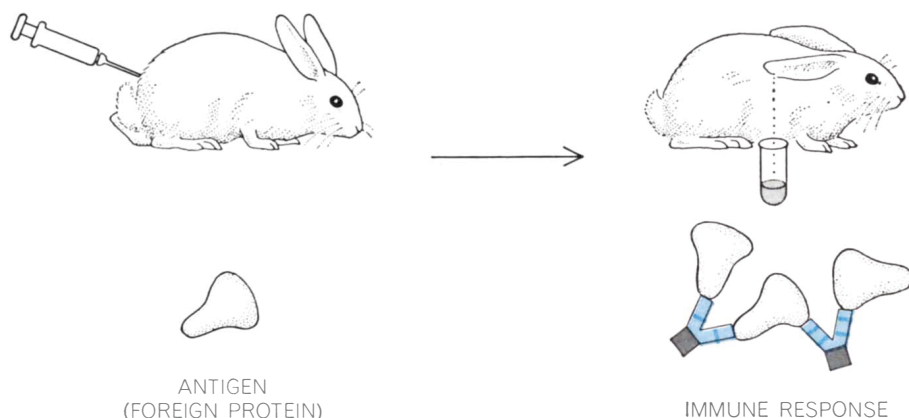
All antibodies are found in a group of related serum proteins known as immunoglobulins. The challenge to the protein chemist lies in the fact that antibody molecules are surprisingly similar even though they possess an enormous range of specific combining power. Although it is clear that there must be significant differences among antibodies, no chemical or physical property has yet been found that can distinguish between two antibody molecules: one able to combine specifically, say, with an aromatic compound such as a benzene derivative and the other with a sugar, although the benzene compound and the sugar have no common structural features. Antibodies of quite unrelated specificity appear to be identical,

within the limits of present experimental techniques, except, of course, in their specific combination with antigen.

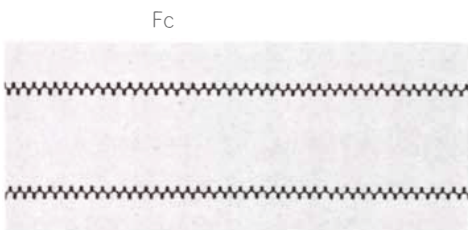
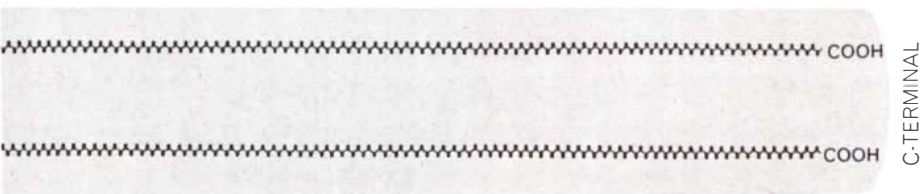
An antibody can be isolated from the serum of an immunized animal only by using the special property of allowing it to combine with the antigen, freeing the complex from the other serum proteins and then dissociating and separating the antibody and antigen. This can be done by allowing a precipitate to form, washing the precipitate well with salt solution and then suspending the precipitate in weak acid. Under these

conditions the antibody-antigen precipitate will dissolve and dissociate, and the antibody and antigen can be separated from each other to yield the purified antibody. As we shall see, however, even this purified material usually contains a variety of antibody molecules that differ slightly in their molecular structure.

If an animal has not been immunized, it will still have a good concentration of immunoglobulin in its blood, usually about 1 percent by weight. This material is believed to be made up of many thousands of different antibodies



**MIXTURE OF SIMILAR ANTIBODIES** can be produced by injecting a rabbit or other animal with a purified antigen, typically a large protein of foreign origin. In response the animal produces antibodies, primarily immunoglobulin gamma, that are able to bind specifically to the antigen. Evidently a given antigen provides many different binding sites, thus giving rise to many different antibody molecules. If blood is removed from the animal



against microorganisms the animal has encountered during its lifetime or against other antigenic substances that accidentally entered its body. Evidence that this view is correct comes from experiments in which small animals have been born and raised in an entirely germ-free environment. Under these conditions the immunoglobulin content of the blood is much lower, perhaps only 10 percent of the immunoglobulin in the blood of a normal animal, suggesting that mild infections are the main source of antigens.

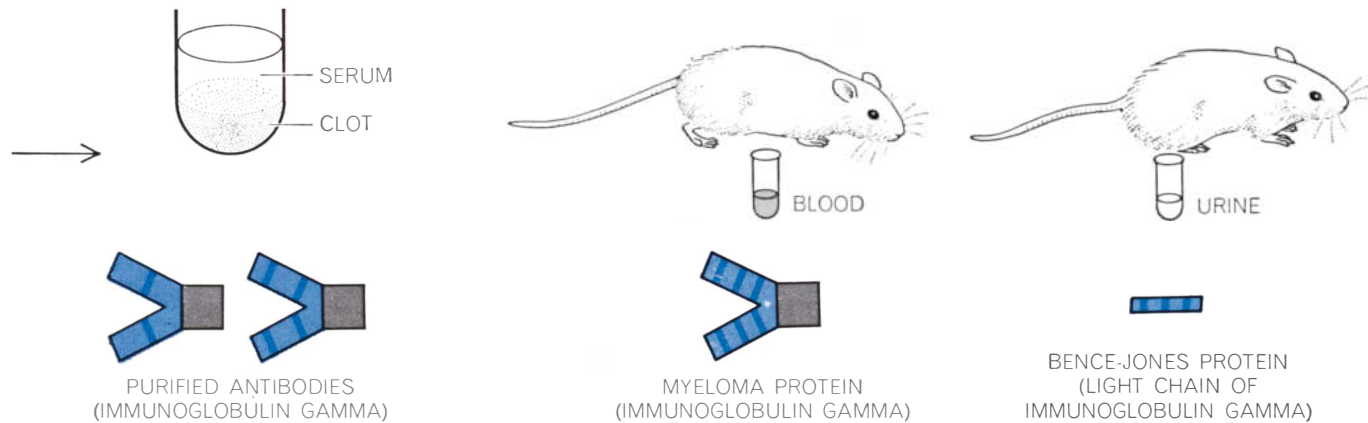
The immunoglobulins can be isolated from serum by the usual methods of protein separation. Hence the protein chemist has available for study two general kinds of immunoglobulin fraction: a complex mixture of many antibodies and purified antibodies that have been isolated by virtue of their specific affinity for the antigen. It would seem to be a relatively straightforward task, after the great progress made in the techniques of protein chemistry in recent years, to carry out detailed studies of such material and pinpoint the differences. Clearly structural differences responsible for the specific combining power of antibodies must exist among them and should become apparent.

Major difficulties have arisen, however, because the immunoglobulins have been found to be a very complex mixture of molecules and the complexity is not necessarily due to the presence of the many different kinds of antibody. One difficulty is that there are three main classes of immunoglobulins distinguished chemically from one another by size,

carbohydrate content and amino acid analysis. Antibodies of any specificity can be found in any of the classes; hence there is no correlation between class and specificity. The class present in the largest amounts in the blood and the most easily isolated is called immunoglobulin gamma. Since most of the work has been done with this material I shall limit my discussion to it.

Immunoglobulin gamma has a molecular weight of about 150,000, corresponding to some 23,000 atoms, of which a carbohydrate fraction forms no more than 2 or 3 percent. Chemical studies have shown that the immunoglobulin gamma molecule is built up of four polypeptide chains, which, as in all proteins, are formed from strings of amino acids joined to one another through peptide bonds. The four chains are paired so that the molecule consists of two identical halves, each consisting of one long, or heavy, chain and one short, or light, chain. The four chains are held to one another by the disulfide bonds of the amino acid cystine [see illustration at top of these two pages]. If the disulfide bonds are split, the heavy and light chains are still bound to each other. If, however, they are put in an acid solution or one containing a substance such as urea, they dissociate and can be separated by their difference in size.

Immunoglobulin gamma molecules can also be split by proteolytic enzymes such as papain, which breaks the molecule into three pieces of about equal size. Two, known as Fab (for "fragment antigen binding"), appear to be identical, and the third, known as Fc (fragment crystalline), is quite different. Fab is so named because it will still combine with the antigen although it will not pre-



and allowed to coagulate, antibodies can be isolated from the serum fraction. Even when purified by recombination with the original antigen, immunoglobulin gamma molecules produced in this way vary slightly.

**IDENTICAL ANTIBODY-LIKE MOLECULES** are produced in large numbers by mice and humans who suffer from myelomatosis, a cancer of the cells that synthesize immunoglobulin. These abnormal immunoglobulins, all alike, can be isolated from the animal's blood (left). Often an abnormal protein also appears in the urine (right). Called a Bence-Jones protein, it seems to be the light chain of the abnormal immunoglobulin.

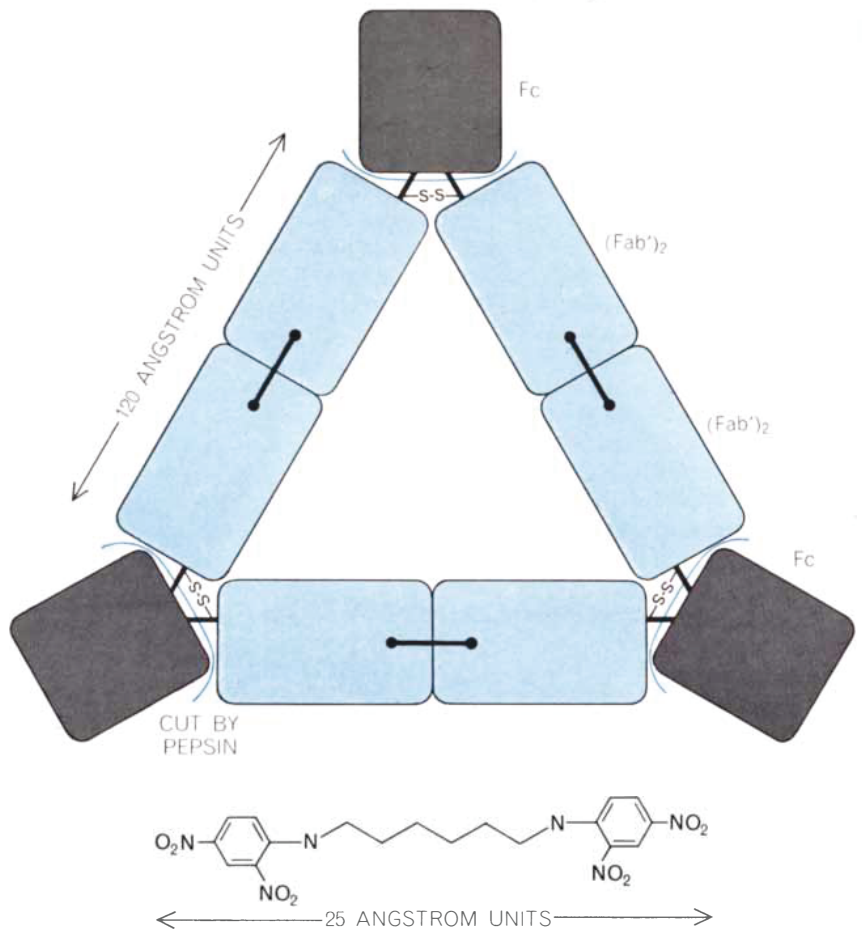
cipitate with it. Each *Fab* fragment carries one combining site; thus the two fragments together account for the two combining sites that each antibody molecule had been deduced to possess. The *Fc* fragment prepared from rabbit immunoglobulin gamma crystallizes readily, but neither the *Fab* fragments nor the whole molecule has ever been crystallized.

Since crystals form easily only from identical molecules, it was guessed that the halves of the heavy chain that comprise the *Fc* fragment are probably the same in all molecules and that the complexity is mainly in the *Fab* fragments where the combining sites are found. The enzyme papain, which causes the split into three pieces, can hydrolyze a

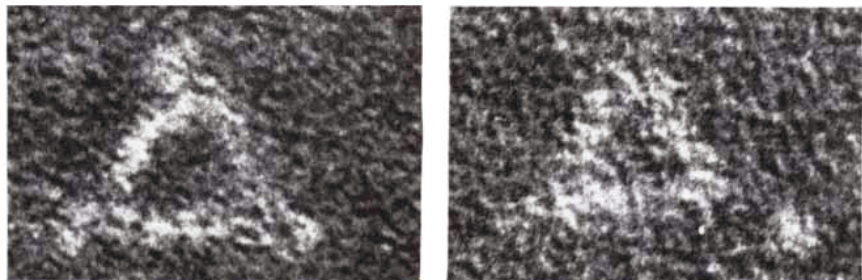
great variety of peptide bonds, and yet only a few in the middle of the heavy chain are in fact split; it looks as if in the *Fab* and *Fc* fragments the peptide chains are tightly coiled in such a way that the enzyme cannot gain access. This suggests a picture in which three compact parts of the molecule are joined by a short flexible section near the middle of the heavy chain.

The full structure of a protein molecule showing the arrangement in space of the peptide chains and the positioning of the amino acids along them can at present only be achieved by X-ray crystallography. Such work has been started at Johns Hopkins University with the *Fc* fragment. Electron microscopy, however, can provide much information about the shape of protein molecules, and successful electron microscope studies have been made recently with rabbit antibodies. When the antibodies are free, no clear pictures are obtained, which suggests that the molecules have a loose structure that is without definite shape. If they are combined with antigen, however, good pictures can be made. Michael Green and Robin Valentine of the National Institute for Medical Research in London prepared antibodies in rabbits that would combine with a benzene derivative known as a dinitrophenyl group. This can be done, as Karl Landsteiner showed many years ago, by injecting into the rabbit a protein on which dinitrophenyl groups have been substituted. Antibodies are formed, some of which combine specifically with the substituent dinitrophenyl coupled onto other proteins or into smaller molecules.

Green and Valentine investigated the smallest compound carrying two dinitrophenyl groups that would cross-link two or more antibody molecules. This proved to be a six-carbon chain with a dinitrophenyl group at each end. This material does not form a precipitate with antibody, but with the electron microscope one can see ringlike structures that appear to contain three to five antibody molecules [see illustrations on page 81 and at left]. The small antigen molecule is not visible. The three-component structure is believed to consist of three antibody molecules linked by three molecules of invisible antigen. The lumps protruding from the corners are thought to be *Fc* fragments. This interpretation is supported by using the proteolytic enzyme pepsin to digest off the *Fc* fragment, leaving two *Fab* molecules held together by a disulfide bond and referred to as  $(Fab')_2$ . When these  $(Fab')_2$  molecules are combined



ANTIBODY-ANTIGEN COMPLEX seen in electron micrographs (below and page 81) is thought to have this triangular structure. Below it, drawn to a large scale, is the synthetic antigen: a six-carbon chain carrying a dinitrophenyl group at each end. Three such antigen molecules appear able to bind together three immunoglobulin gamma molecules.



EFFECT OF PEPSIN COMPLEX is demonstrated in electron micrographs taken by Green and Valentine. In the normal complex formed by immunoglobulin gamma and the dinitrophenyl compound (left) a typical triangular structure contains a small lobe, or lump, at each corner, which is thought to be the *Fc* part of the immunoglobulin molecule. If the antibody is first treated with pepsin, which splits off the *Fc* fragment, the remaining  $(Fab')_2$  molecule still reacts with the antigen but the corner lobes are missing (right).

with antigen, rings are formed as before, but the lumps at the corners are now gone, confirming the idea that they were indeed the *Fc* part of the molecule.

Since most interest centers on the antibody combining site, the next problem to solve is whether the site is to be found in the light chain, which is entirely in the *Fab* fragment, or in the half of the heavy chain that is also present, or whether the site is formed by both chains together. It has not been possible to get a clear answer to this problem because the chains cannot be separated except in acid or urea solutions; this causes a partial loss of the affinity for antigen, which is not recovered even after the acid or urea is removed. Present evidence suggests that the heavy chain is the most important but that the light chain plays a role. This may be because it actually forms a part of the site or because it helps to stabilize the shape that the heavy chain assumes and hence plays a secondary role that may be only partially specific.

In any case, the field is clear for a direct attempt at comparative studies of the chemical structure of the light chain as well as of the half of the heavy chain that lies in the *Fab* part of the molecule. The shape and hence the specificity of the combining site must depend on the configuration of the peptide chains of the *Fab* fragment; this is believed to be determined only by the sequence of the different amino acids in the chain. Therefore it is reasonable to expect that if the amino acid sequence is worked out for the *Fab* half of the heavy chain and perhaps also for the light chain, then in some sections sequences will be found that determine the configuration of the combining site and that will be characteristic for each antibody specificity. Attempts to carry out such sequence studies, however, seemed unattractive because of convincing evidence that all preparations of immunoglobulin gamma—even samples of purified antibodies obtained by precipitation with a specific antigen—were actually mixtures of many slightly different molecules with presumably different amino acid sequences.

Although the complexity of immunoglobulin gamma (and of the other classes of immunoglobulins) has presented investigators with a most difficult puzzle, considerable progress has now been made in solving much of it [see illustration on this page]. First, there are two kinds of light chain, named kappa and lambda, but in any one molecule both light chains are of the same type.

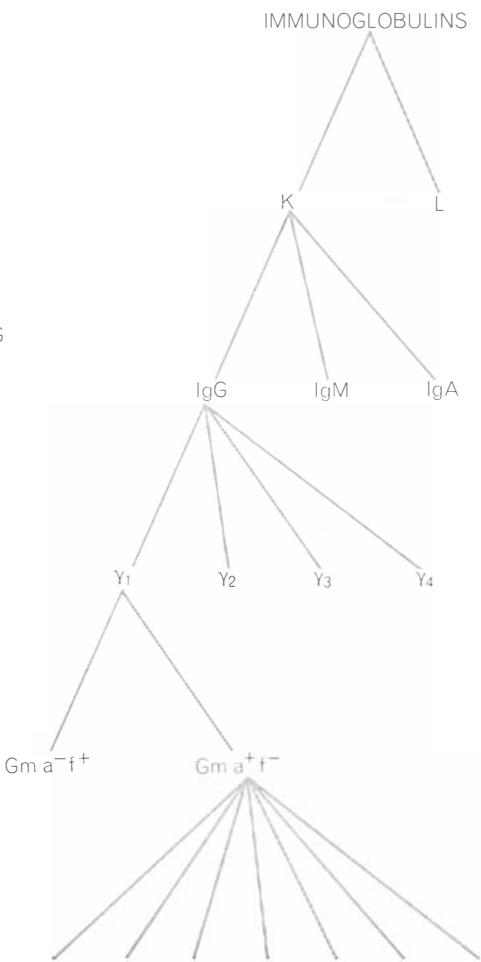
TWO TYPES DEPENDING ON STRUCTURE OF LIGHT CHAIN

THREE MAJOR CLASSES DEPENDING ON STRUCTURAL DIFFERENCES IN HEAVY CHAIN

FOUR SUBCLASSES DEPENDING ON CHANGES IN STRUCTURE OF HEAVY CHAIN

INHERITED ALLELIC VARIANTS OF EACH HEAVY OR LIGHT CHAIN

UNKNOWN NUMBER OF IDIOTYPIC FORMS



**SUBDIVISIONS OF HUMAN IMMUNOGLOBULIN** presented investigators with a difficult problem to unravel. For simplicity, subdivisions are shown for only one branch at each level. The abbreviation “*IgG*” stands for immunoglobulin gamma, the antibody found in largest amounts and the one most easily isolated. Idiotypic forms are apparently unique to individual animals and may involve alterations in both the light and the heavy chains.

The molecules containing kappa chains are known as *K* type and those with lambda chains as *L* type. Then in some species (probably in all) the immunoglobulin gamma class contains several subclasses; four have been identified in human gamma globulin. The subclasses differ in their heavy chains, which carry not only the characteristic features of the class but also small differences that distinguish the subclasses. In any one individual, molecules will be found of both *K* and *L* type, and they belong to all the subclasses. In addition each of the kinds of chain shows differences, known as allelic forms, that are inherited according to Mendelian principles. In an individual homozygous for this property only one allelic form of, say, the kappa chain will be present, but in a heterozygous individual there will be two forms of the kappa chain. It scarcely need be stressed that all these phenomena lead to a very complex mixture of molecules of immunoglobulin gamma in the serum of any

individual. Yet there is still another kind of complexity termed idiotypic. In certain circumstances it is possible for an animal to synthesize antibody molecules that are unique to itself, distinct from other antibody molecules of the same specificity in other individuals of the same species—and distinct from all other immunoglobulins in its own blood. Perhaps the most remarkable aspect of all of this is that the complexity seems to bear no relation to the structure of the antibody combining site. As far as we know at present, any antibody specificity may be found on any of these many different kinds of molecule. All such variations are likely to be based on differences in amino acid sequence, and already some differences relating to subclass and allelic changes have been identified. The structural differences are so small, however, that it is not possible to separate out single kinds of molecule by the methods available for the fractionation of proteins.

Thus it was a great step forward when it was recognized that in certain forms of cancer, immunoglobulin molecules of apparently a single variety appear in the blood. Such immunoglobulins have only one type of light chain and one subclass of heavy chain, and each chain belongs to one or the other allelic form. As far as we know each chain has only one amino acid sequence and therefore belongs to only one idiotypic form.

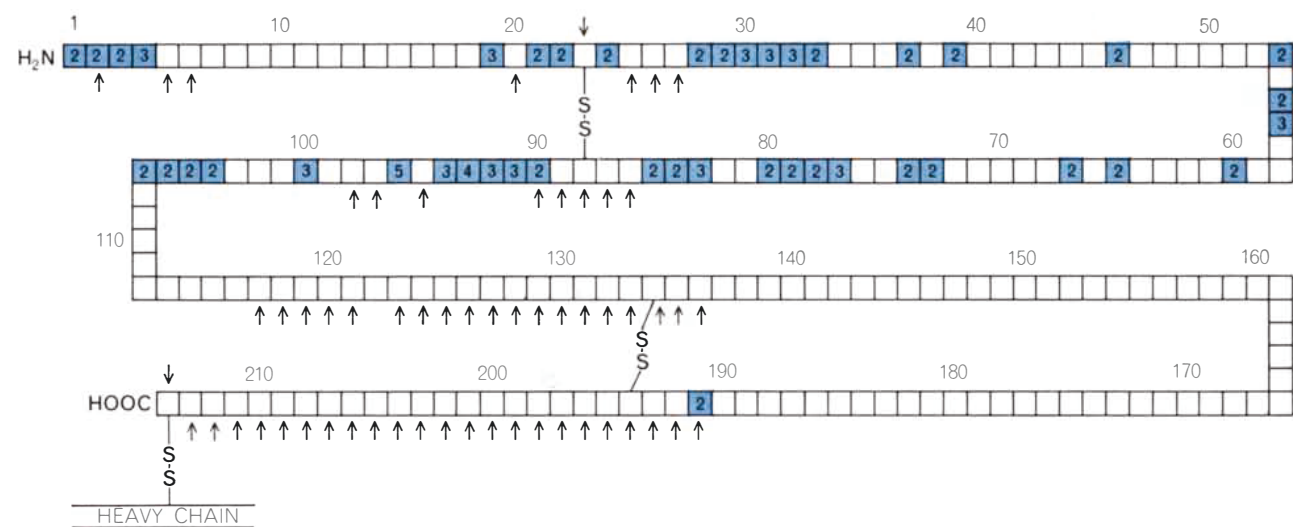
The disease responsible for this unique production of antibody is known as myelomatosis. Observed in both mice and men, it is a cancer of the cells that synthesize immunoglobulin, often those in the bone marrow. Apparently a single cell, one of the great number that synthesize immunoglobulins, starts to divide rapidly and leads to an excessive production of a single kind

of immunoglobulin. This provides evidence, incidentally, that the complexity of immunoglobulin molecules arises from their synthesis by many different kinds of cells. These abnormal immunoglobulins are known as myeloma proteins. Because they are often present in the blood in a concentration several times higher than all the other immunoglobulins together, they can be isolated rather easily.

Moreover, in about half of all myeloma patients an abnormal protein appears in the urine in large amounts. This substance was first observed by Henry Bence-Jones at Guy's Hospital in London in 1847 and has been known ever since as Bence-Jones protein. Its nature, however, was not recognized until five years ago, when Gerald M. Edelman and J. A. Gally of Rockefeller University and independently Frank W.

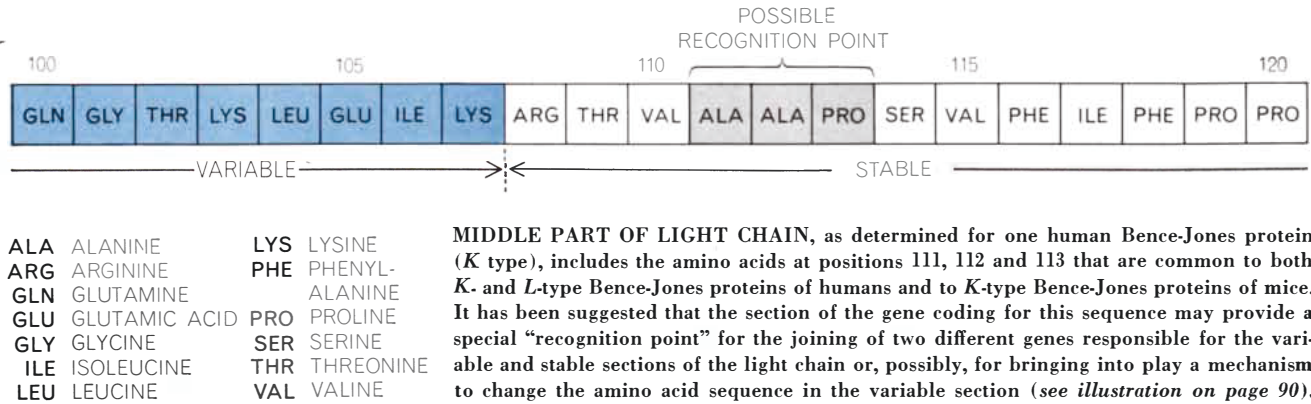
Putnam of the University of Florida showed that Bence-Jones protein is probably identical with the light chains of the myeloma protein in the serum of the same patient. Because Bence-Jones proteins can be obtained easily, without any inconvenience to the patient, they were the first materials used for amino acid sequence studies.

Although complete sequences have been worked out for only two Bence-Jones proteins in the mouse and only three human Bence-Jones proteins, perhaps 20 more have been partially analyzed. A remarkable fact has emerged. It seems that all Bence-Jones proteins of the same type have exactly the same sequence of amino acids in the half of the molecule that ends in the chemical group COOH (hence known as the C-terminal half) but show marked variation in the half that ends in the group



**IMMUNOGLOBULIN LIGHT CHAIN**, represented by analyses of human Bence-Jones proteins of the *K* type, has 214 amino acid units. Colored squares show where amino acids have been found to vary from one protein to another; blank squares show where no variation has yet been found. Numbers in the squares indicate how many different amino acids have been identified so far at a given site. Arrows mark positions where a particular amino acid has been found in at least five different proteins. Complete amino acid

sequences are now known for three human Bence-Jones proteins and partial sequences for about 20 others. All variations occur in the first half of the chain with one exception, the variation at position 191. This is related to the allelic, or inherited, character of light *K* chains, hence differs from the alterations in the variable half of the chain. The diagram is based on one recently published by S. Cohen of Guy's Hospital Medical School in London and C. Milstein of the Laboratory of Molecular Biology in Cambridge.



|     |               |     |                    |
|-----|---------------|-----|--------------------|
| ALA | ALANINE       | LYS | LYSINE             |
| ARG | ARGININE      | PHE | PHENYL-<br>ALANINE |
| GLN | GLUTAMINE     | PRO | PROLINE            |
| GLU | GLUTAMIC ACID | SER | SERINE             |
| GLY | GLYCINE       | THR | THREONINE          |
| ILE | ISOLEUCINE    | VAL | VALINE             |
| LEU | LEUCINE       |     |                    |

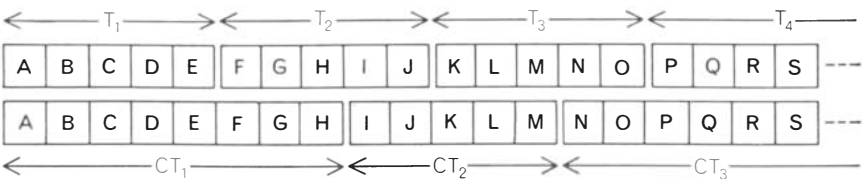
**MIDDLE PART OF LIGHT CHAIN**, as determined for one human Bence-Jones protein (*K* type), includes the amino acids at positions 111, 112 and 113 that are common to both *K*- and *L*-type Bence-Jones proteins of humans and to *K*-type Bence-Jones proteins of mice. It has been suggested that the section of the gene coding for this sequence may provide a special "recognition point" for the joining of two different genes responsible for the variable and stable sections of the light chain or, possibly, for bringing into play a mechanism to change the amino acid sequence in the variable section (see illustration on page 90).

NH<sub>2</sub> (the N-terminal half). Of 107 amino acid positions in this half, at least 40 have been found to vary. No two Bence-Jones proteins have yet been found to be identical in the N-terminal half, so that the possibility of molecular variation is clearly great. Given the possibility of variation at 40 sites and supposing that only two different amino acids can occupy these sites, it would be possible to construct 2<sup>40</sup>, or more than 10 billion, different sequences. Actually as many as five different amino acids have been found to occupy one of the variable sites [see upper illustration on opposite page].

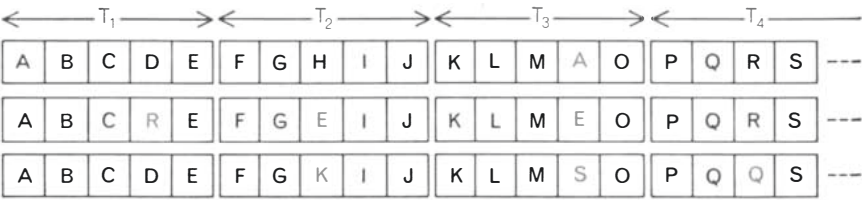
The amino acid sequence studies of the heavy chain are less advanced than those with the Bence-Jones proteins because the material is more difficult to obtain and is more than twice the length. Results with the heavy chain of two human myeloma proteins, however, have shown them to have many differences in sequence for more than 100 amino acids from the N-terminal end, whereas the remainder of the chain appears to be identical in both cases. Accordingly it seems certain that the heavy chains will show the same phenomena as the light chains; it is possible that the length of the variable section in both chains will be similar.

Inasmuch as both variable sections are in the Fab fragment of the molecule it seems obvious that these sections must participate in creating the many different antibody combining sites. All the work discussed here has been done with myeloma proteins, and since each has a single amino acid sequence in both heavy and light chains, it would follow that each will be a specific antibody against one of an untold number of different antigenic sites. The chances, therefore, of finding a myeloma protein in which antibody specificity is directed to a known, well-defined antigenic site seemed small. Nevertheless, several myeloma proteins have recently been found to possess antibody-like activity against known antigens. A comparison of the sequences of their heavy and light chains may give a lead as to where the combining site is located.

It has been believed with good reason that myeloma proteins are typical of normal molecules of immunoglobulin gamma, each being a homogeneous example of the many different forms present. It thus seemed likely that any attempt to determine the amino acid sequence of immunoglobulin gamma from a normal animal would be impossible, especially in the variable region that is



**DETERMINATION OF AMINO ACID SEQUENCE** in the polypeptide chains of proteins depends on the use of enzymes that cleave the chains into short fragments next to particular amino acids. The sequence in the resulting fragments can then be established. Thus trypsin might split a chain into fragments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. Another enzyme, chymotrypsin, might split the same chain into fragments CT<sub>1</sub>, CT<sub>2</sub> and CT<sub>3</sub>. Since these fragments must overlap one can establish their order unequivocally and thereby the sequence of the entire chain.



**IMMUNOGLOBULIN SEQUENCE** should be amenable to analysis even though a particular antibody sample might contain a variety of slightly different molecules. Slight variations at certain positions (color) should not prevent the ordering of similar fragments.

of particular interest. One would expect normal immunoglobulin gamma to be a mixture of many thousands of different molecules, each with a different sequence in the variable region.

Amino acid sequences of polypeptide chains are found by using enzymes to break the chains into pieces from 10 to 20 amino acids long. It is then possible to work out the sequence of each piece. By using different enzymes the original chain can be broken at different places, with the result that some pieces overlap. This provides enough clues for the whole sequence to be put together, rather like a one-dimensional jigsaw puzzle [see upper illustration above]. When the protein is pure, there is only one order of amino acids possible, and all the sequences of the individual fragments will fit into it.

One can see that if this method were attempted with a protein that was in fact a mixture of many slightly different proteins, each with a different sequence, a hopelessly confusing picture would probably result. The work with the myeloma protein suggested, however, that there would be a constant part as well as a variable part, and it seemed worthwhile to see what progress could be made in determining at least the constant part. Work at Duke University showed that the whole of the Fc section of the heavy chain of normal rabbit immunoglobulin gamma gave a coherent sequence and was therefore part of the stable section, as had been expected. Recent work in our laboratory has now

shown that the coherence continues well into the other half of the heavy chain. Although the work is far from complete, it seems possible that a full sequence will be established right through the entire heavy chain. Variations have been picked up in a number of positions and no doubt many more will be found, but the results are not completely confusing, as might have been expected if normal immunoglobulin gamma were a mixture of many thousands of myeloma proteins, each with substantially different sequences in the variable parts of the chain. The conflict between the results with the myeloma proteins and the recent results with normal rabbit immunoglobulin may be more apparent than real.

What does all this mean in terms of the structure of antibodies and their power to combine specifically with antigens? The phenomenon of a variable section and a stable section in both heavy and light chains is extraordinary and is unique to immunoglobulins; the variable section is in the part of the molecule known to contain the combining site. It therefore seems certain that this must be the basis of the specific configuration of the combining site.

It should be emphasized that all this work is very incomplete. In another year or so it will undoubtedly be much easier to see just how different one myeloma protein is from another in both the heavy and the light chains. It may be that the differences between any two

*KLH launches an inquiry into "Subjective Value."*

# (SURVEY)

## Question #1

How much would you pay to keep your wife one more year?

DO YOU REMEMBER the game kids used to play in school where you were asked how much money it would take to get you to sell your country's secrets? (Assuming no torture.) Or your dog?

It was a way of thinking about the value you *really* placed on a thing.

One of the first things you learned was that "features" had very little to do with it. (For example, if your country had had 20 more rivers, or your dog's tail wagged at 86 Per Minute—six less than an "average" dog's—the answer would hardly have changed.)

When KLH began making stereo equipment ten years ago, our founders (K., L., and H.) noticed that grownup manufacturers talked as though features had *everything* to do with value. We hated that. We still do.

"Feature:" Injecting 380 horsepower into cars that have no plausible market save those who commute back and forth over the Bonneville Salt Flats.

Or Again: Advertising 300 watts of power in a high priced stereo console unit to give it the *appearance* of value. (Neglecting to mention that large numbers of watts have nothing to do with hearing the music accurately, or even loudly, both of which depend on what kind of equipment you've squeezed the watts

into. 35 watts in good equipment will do far better.)

42-22-36

To define worth solely in terms of features is like determining the "market value" of a wife from her height, age, weight, width of smile, tendency to suntan evenly, and the number of pounds of food she is capable of cooking up in an evening.

It's true enough we all like to have *something* explicit to help our thinking. Even Consumer Reports will sometimes find itself detailing competitive features and statistics; akin to Playboy's 42-22-36 ratings.

But studying the centerfold and accompanying data simply doesn't give us all the information we really need. What does? Well, probably nothing short of a few years in the same house together.

### BASEBALL PLAYER

Packard had it right, way back in the twenties, when its advertising rested on the confident slogan "Ask The Man Who Owns One."

(It was a new kind of "testimonial" but it's been watered down since. How is a man today to depend on testimonials to choose, say, his cigarette brand when dozens of equally beloved baseball players can't get together about which is best?)

Still, the principle of determining value through testimonials makes very good sense:

Economists, for instance, say value can be understood as “some measure of the sense of loss one experiences after being deprived of a commodity or service,” or, ask the man who owns one how much he’d dislike losing it. (The boy contemplating his dog’s worth figured it out the same way.)

Any other way of measuring value, like establishing a ratio between features and price, is at best only a guess, made *before* anyone could possibly know.

DEPRIVED OF YOUR WIFE

What we propose, then, is a technique of *measuring* the sense of loss as a way of thinking about “Subjective Value,” i.e., what a commodity means to someone who has it.

So. Assume for a moment that you are about to be deprived of your wife. (Substitute

husband or “good friend” where applicable.) How much would you pay in dollars to keep her one more year? When you’re through thinking about that one, fill in No. 1 and have a look at the rest of the questionnaire.

You see what we’re up to here.

We began on this idea because we already have evidence (based upon a comparison of the number of hours owners sit listening to KLH phonographs as opposed to other brands) that our \$300 stereo system is cherished somewhat more than at least one \$400 system we could name; and perhaps twice as much as another \$300 set.

Doubtless the same situation exists among magazines—some are surely valued more than others—or sewing machines, or autos, or toothpaste. Toothpaste? Well, we’ll soon see, and if you’re interested we will be pleased to let you know what we learn.

# QUESTIONNAIRE

If one or more of these questions interests you, then kindly fill in the blank spaces that apply and mail to the address we have listed at lower right. For our part, we will gladly send you a tally of the results of this questionnaire, and others we are doing in subsequent ads, if you also add your name and address. Thank you.

- 1 (See Headline).
- 2 Are you a subscriber to this publication? If not, do you read every issue of it? If your answer is yes to either of these, and you were informed that because of financial difficulties the publication might discontinue publishing, how much would you be willing to pay for one more issue rather than be deprived of it? One more year’s subscription?
- 3 Do you have telephone service at home? If yes, assume you now pay an average of \$20 monthly for this service. How much additional would you pay, rather than be deprived of it?
- 4 The automobile you now own was purchased in what year? At what price? What make? Assuming it’s in good running order, and that you couldn’t get another one like it, how much would you pay to keep it during the upcoming year?
- 5 Do you own a piano? What kind? How much did you pay for it? How long ago? How much would you pay to keep from being deprived of it?
- 6 Do you regularly use a particular brand of toothpaste? If yes, which brand? Assuming you were informed that because of financial difficulties your brand of toothpaste might go out of

business. How much would you be willing to pay, above its present cost, to have one more tube, rather than be deprived of it?

- 7 Assume for the moment that an offer was being made for your wife’s wedding dress. How much would you be willing to sell it for? What does your wife say?
- 8 Do you own stereo equipment at home? A console? A one-piece table model? A three-piece system? Components? Which make(s)? How much did it cost you to buy? How long ago? If you were about to be deprived of the set you now own, and knew you could not get another of the same kind, how much would you be willing to pay to keep it?

(If you worry that by putting your name below you may be subjecting yourself to a barrage of KLH literature, or that we may send a salesman around, or sell your name to some “list house,” rest easy. We won’t. Though if you would like to have a catalog and the name of the store near you that sells our equipment, please so indicate in the appropriate box.)

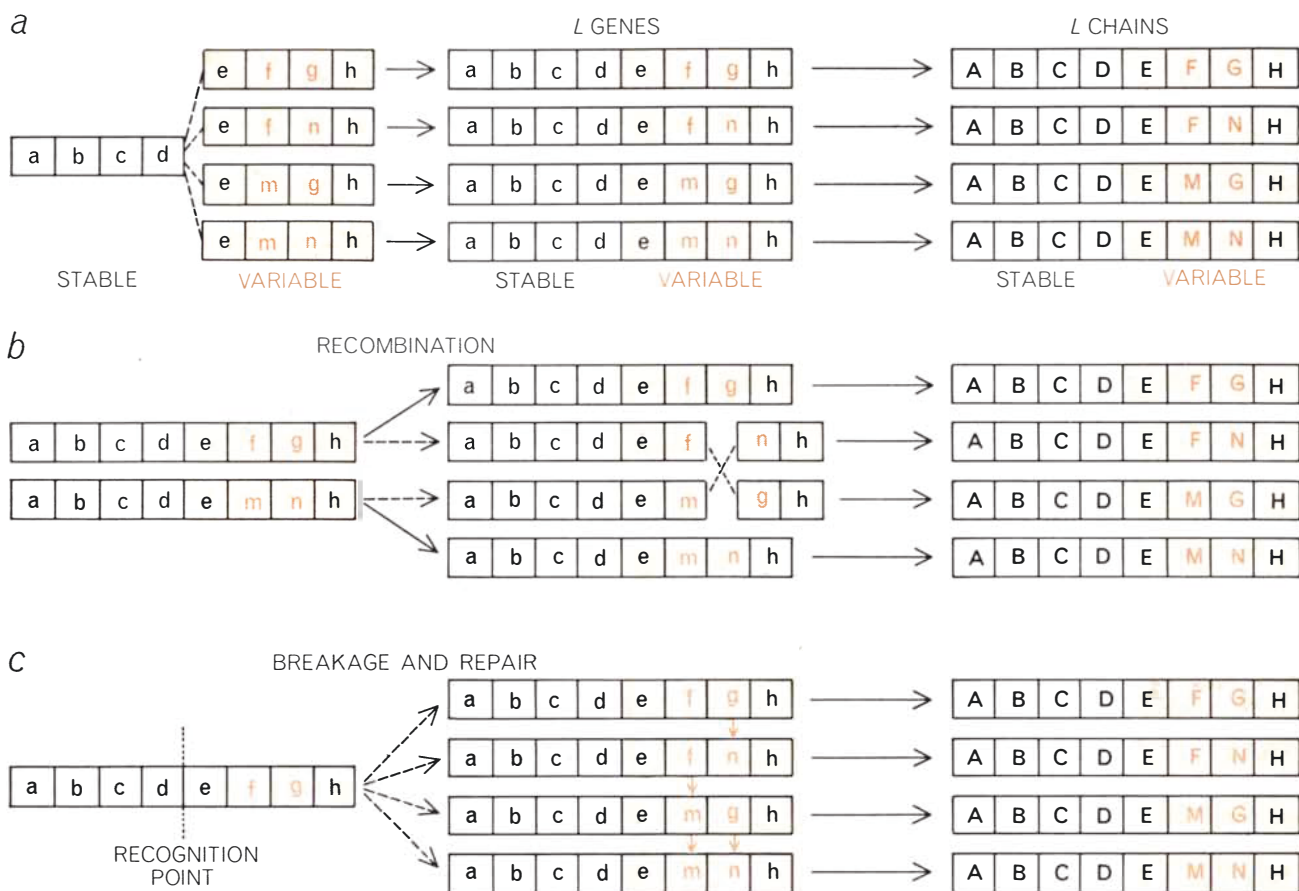
Name

Address

CityStateZip

☐ Please send catalog ☐ Forward survey results

Mail to: Henry M. Morgan, Pres., KLH Research and Development Corp., 30 Cross St., Cambridge, Mass. 02139



**VARIABILITY OF IMMUNOGLOBULIN MOLECULES** has been explained by three principal hypotheses. The simplest (a) suggests that one gene codes for the stable section of each chain and that a great number, perhaps hundreds of thousands, code for the variable section. A second idea (b) is that several genes are divided into stable and variable sections and that the latter inter-

change parts during cell division. A third proposal (c) suggests that there may be a recognition point in the gene (see lower illustration on page 86) and that an enzyme partially splits, or breaks, the gene on the variable side of that point. When repaired by other enzymes (arrows), mistakes are made, thus giving rise to many different amino acid sequences in the antibody molecule.

will on the average be small, so that for a mixture of many molecules the amino acid in any one position will be common to 80 or 90 percent of the molecules [see lower illustration on page 87]. Presumably this explains how it is possible to find a comprehensible amino acid sequence in normal immunoglobulin gamma.

It may also be, however, that myeloma proteins are not quite typical of normal antibodies. Because they are the result of a disease they may exaggerate a normal phenomenon. Although they are invaluable in drawing attention to a fundamental mechanism, they may mislead us by exhibiting greater variability than is present in normal immunoglobulin gamma.

Whatever the answer, the existence of a stable section and a variable section, which has been shown so clearly in the Bence-Jones proteins and which also occurs in the heavy chains, is a remarkable phenomenon. The mechanism of its biological origin has aroused intense interest. Many hypotheses have been put forward, but there are perhaps three

principal ones [see illustration above].

A straightforward mechanism would be to have a single gene coding for each stable section in the antibody molecule and as many genes as necessary (tens of thousands or hundreds of thousands) coding for the variable sections. The cell would also be provided with a means for fusing the product of the two kinds of gene to construct the complete immunoglobulin molecule. (In this as in the other suggestions, the presence of an antigen would somehow trigger production of the appropriate antibody.)

A second proposal invokes the concept of genetic recombination, which involves the exchange of parts of genes. One can imagine several genes that are divided into a stable portion and a variable portion. During cell division, when genes are pairing and duplicating, the variable portion would interchange sections, thereby giving rise to many different genes capable of coding the variable parts of the antibody molecule.

The third suggestion visualizes that the gene for, say, the light chain may

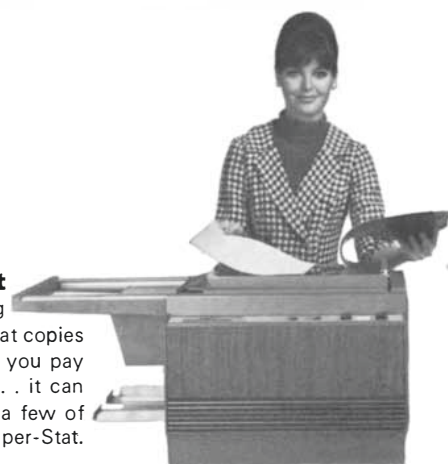
contain a "recognition point" midway in its structure [see lower illustration on page 86]. This might provide a specific attachment site for an enzyme that can split the nucleic acid of the gene only on the side coding for the variable section. When the broken portion is repaired by other enzymes, mistakes are made, thereby giving rise to many different sequences of nucleotides—the nucleic acid building blocks that embody the genetic message. These differences are then translated into different amino acid sequences in the variable portion of the antibody molecule.

There is no clear answer as to which methods, if any or all, are the operative mechanisms, but a continuation of the structural studies may provide a clearer understanding. When this understanding is attained, it should lead to ideas about how to change, stimulate or suppress immune reactions as medical practice requires and therefore should be of great practical value as well as solving one of the most intriguing problems in biology.



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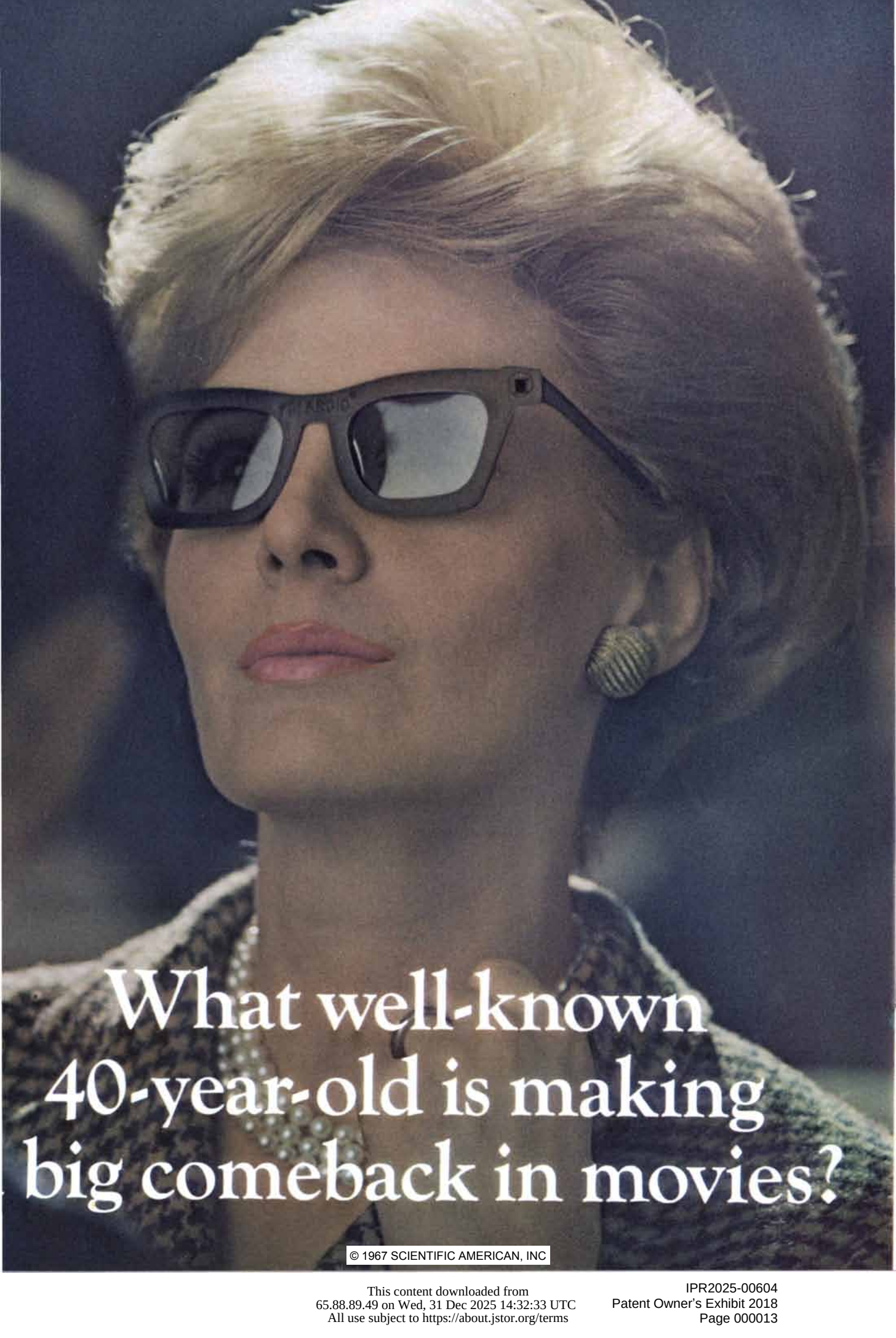
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**APÉCO** AMERICAN PHOTOCOPY EQUIPMENT COMPANY / 2100 WEST DEMPSTER STREET, EVANSTON, ILLINOIS 60204

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What well-known  
40-year-old is making  
big comeback in movies?

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The 40-year-old isn't the lady. It's the glasses she's wearing. They contain Polaroid linear polarizing filters which were invented in 1927.

Each filter contains a molecular grid which excludes all light rays except those which vibrate perpendicularly to the bars in the grid. This characteristic has given polarizing filters a wide range of uses in science and industry.

In the 1950's the Polaroid filter found a new commercial application. It made 3-D movies in color possible. Here's how the process worked:

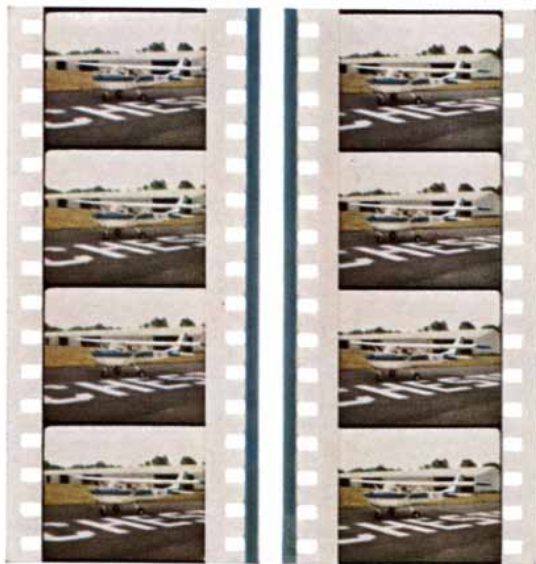
Perception of depth starts from the fact that the right eye point of perspective is different from the left eye point of perspective. The brain translates this difference into perception of a three-dimensional world.

To duplicate this phenomenon, the 3-D movies of the 1950's were made by filming a scene simultaneously with two cameras (placed to correspond to the right and left eyes of a viewer).

The resulting right and left images were then superimposed on the movie screen through a pair of movie projectors.

In order to code the right and left images for the respective right and left eyes of the audience, the right image was projected through a polarizing filter having its polarizing axis at 45 degrees clockwise from the vertical, and the left image was projected through another filter with its polarizing axis 45 degrees counterclockwise. The screen had an aluminized surface so that it did

*Old technique: left eye and right eye images on their respective filmstrips.*



not depolarize the light.

Polarizing stereo glasses with lenses oriented right and left to correspond to the right and left projection filters were placed over the eyes of the viewer to decode the image on the screen. Thus, the right eye saw only what was "seen" by the right camera, and the left eye saw only what was "seen" by the left camera.

*New technique:  
left eye and right eye  
images alternating on  
a single filmstrip.*



Under ideal conditions, 3-D gave an excitingly realistic illusion of depth to the screen.

Unfortunately, conditions were seldom ideal. It was almost impossible to maintain perfect synchronization and registration of images with two films running through two projectors. And 3-D was more apt to give eyestrain than pleasure.

But now there's a new 3-D process, SPACE-VISION (utilized in "The Bubble," a film by Arch Oboler). The right and left images are photographed through two lenses as before. However, through the use of prisms, the images are recorded one above the other on a single strip of film. The film is then projected through another set of prisms and lenses which superimpose the right and left images on the screen.

With both images on one film, and with prisms and lenses in fixed positions, synchronization and registration can be perfect.

In addition, the proportions of this image (as compared with the old 3-D image) provide the widest possible picture on the screen. This extends the picture to the area of the viewer's peripheral vision and further enhances the illusion of depth.

Coding and decoding the right and left images is still accomplished with the kind of Polaroid polarizing filters that made the first 3-D movies possible.

Our present viewers are slightly larger than their predecessors (to encompass the wider screen). And the original paper frames have been replaced with plastic (so they fit comfortably, even over eyeglasses).

Those are the only improvements they needed. (How many other 40-year-olds in show business hold up that well?)

**POLAROID CORPORATION**

CAMBRIDGE, MASS.