Immunological probes in cardiovascular disease*

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SUMMARY The immune system has long been recognised as playing a central role in the organism's defence against infectious diseases and possibly the development of neoplasia. The active stimulation of the immune system by immunisation and the passive administration of antitoxins have a venerable history in medicine. Yet the concept that antibodies may be used to modify physiological or pharmacological effects or may act as diagnostic agents in the living organisms has only recently come to be recognised. Advances, both in an understanding of the structural chemistry of the antibody molecule and in the ability to culture antibody-producing cells, now permit the selection and production of homogeneous antibodies and their smaller fragments in quantity by means other than conventional immunisation. These innovations will allow the development of a new pharmacology based on the remarkable resolving power of the antibody combining site. Antibodies or their fragments are shown to inhibit the pressor action of renin, to neutralise the pharmacological actions of digitalis, to block the beta-adrenergic receptor, and to detect and image myocardial infarcts.

Antibodies represent a set of molecules that exhibit extraordinary specificity and selectivity. This has been appreciated since the work of Landsteiner,¹ who, in the early years of this century, was able to provide examples such as the ability of an antiserum to resolve the d and l stereoisomers of a simple aliphatic molecule, tartaric acid. More complex organic compounds that vary only by a single substituent group (such as the steroids, or digoxin and digitoxin, which differ in one hydroxyl group) may be readily separated.² The general structure of the antibody molecule is remarkably conserved, so that it is difficult for the chemist to tell the difference between two antibodies of varying specificity. There is a small region of the molecule, however, that is highly variable in its structure (the complementarity region) and makes up the surface that binds antigen. It is the amino acid sequence in this complementarity region that determines the nature of the antigen recognised. Recent work has uncovered the mechanisms responsible for variation in the amino acid sequence of the complementarity region.

The antibody molecule comprises four polypeptide chains, two identical light and heavy chains. The variable region of the light chain is the product of two

genes: V, which occurs in several hundred copies, and J, of which four copies have been identified.³⁻⁶ V and J of the light chain may occur in any combination. The heavy chain's variable region is the product of three genes: V, in several hundred copies, D, in 10 or more copies, and J, in four copies.^{7,8} As in the light chain, any permutation and/or combination of these genes may occur. These mechanisms alone account for 107 different antibodies. In addition, somatic-point mutation has been shown to occur. Thus the number of possible antibodies must actually exceed 10¹⁰.

Clinicians have not neglected the immune system, but its deliberate manipulation has been restricted to a limited number of areas. Of course, immunisation has long been a cornerstone of preventative medicine. The passive infusion of pneumococcal-specific antisera had a brief vogue in pre-antibiotic days, while tetanus and Rh antibodies are still of great value today. By providing a method for measuring substances in biological fluids that would not be possible by any other means, the immunoassay has produced a literal explosion in the use of antibodies as *in vitro* diagnostic agents. Yet, aside from these few uses, this remarkable group of compounds has been largely neglected in its potential utility as a source of pharmaceutical agents with both diagnostic and therapeutic applications.

There may be several explanations for this neglect.

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Before very recent times, antibodies were only available as complex mixtures of proteins, isolated in very limited quantities from animal sera. Standardisation of an antibody is not possible since each immunised animal provides an antiserum of different properties. There is even variation among specimens of sera of single animals at different times after immunisation. An additional problem is the potential immunogenicity of heterologous antibodies in the recipient. Each of these problems has been overcome by application of a new technology that has only been available in the past several years. Antibodies may now be produced by cell culture methods in infinite quantities with previously unimagined homogeneity and reproducibility.⁹ Antibody fragments may be created that reside within the body for shorter periods of time and are less likely to provoke an immune response.¹⁰ We are on the threshold of producing human antibodies by the same cell-culture approach.^{11 12}

I shall review the potential application of antibodies to human pharmacology in four different areas: inhibition of the action of a hormone that is secreted in excess, blockade of a physiological receptor, detection of a newly exposed antigen in the living organism, and neutralisation of the toxic effect of a drug.

Blockade of physiological action of renin by specific antibody

Though renin has been known to play a role in circulatory control since the classic work of Goldblatt *et al.* in the 1930's,¹³ its precise importance in a number of specific circumstances has been in doubt. Much has been learned from the application of inhibitors directed at several of the steps in the sequence leading to the production and action of the final product of renin, angiotensin II.¹⁴ Most of these compounds lack specificity.¹⁴ The competitive inhibitors, such as saralysin, of angiotensin II's action on receptors are partial agonists. The angiotensin-converting enzyme is identical to the enzyme that inactivates bradykinin,¹⁵ and thus its inhibitors also affect the kinin system. In addition, compounds such as captopril have been shown to stimulate prostaglandin synthesis.¹⁶ Since both the kinin and the prostaglandin systems have an effect on vasoregulation, it is difficult to define the specific part that renin plays in interpreting experiments in which these inhibitors are used. A specific antibody for renin should be a highly selective antagonist. Renin antibodies have been used as physiological reagents for many years,^{17 18} yet their specificity has been in doubt since we now know that the preparations then used as immunogens contained less than 1% of the enzyme.

Dzau *et al.*¹⁹ in our laboratory purified canine renin some 600 000-fold in an eight-step process that

yielded a product homogeneous by several criteria. Antibodies specific for purified canine renin produced in a goat inhibited the pressor action of the enzyme but did not modify the capacity of either angiotensin I or II to raise blood pressure.²⁰ This antibody preparation did not have any effect on the haemodynamics of the unanaesthetised, sodium-replete dog, while a significant hypotensive effect was noted in the sodium-depleted dog when the immunoglobulin G fraction from an antiserum was injected intravenously. Parallel to the fall in blood pressure, a decrease in both plasma renin activity and angiotensin II concentrations was observed, indicating that the antibody was exerting its effect by inhibiting the enzymatic action of renin on its substrate.

Intact antibody has a number of troublesome properties when used as a drug. When the source is a heterologous species, it is an immunogen. After the first use, an immune response develops that may result in anaphylaxis, serum sickness, or at best accelerated elimination. Pharmacological effects may be very persistent, because antibody is eliminated either by metabolism or by the immune system if sensitisation has occurred. The half-life for endogenous immunoglobulins may be measured in days or weeks depending on the species studied, the immunoglobulin's isotype, and whether or not the recipient has been immunised. When there is concern about renal function, the presence of immune complexes is likely to cloud interpretation. In haemodynamic studies, vasoactive peptides released by activation of complement may have independent effects, particularly on haemodynamics.

Some of these problems may be minimised by producing antibody fragments. Immunoglobulin molecules that bind two moles of antigen per mole may be cleaved to smaller molecules by the enzyme papain.²¹ This results in two Fab that each bind one mole of antigen and one Fc that contains the complement binding site. Fab and Fc may be separated readily. Fab has a number of desirable properties when compared with the intact molecule, IgG: equilibrium distribution in extracellular fluid is achieved more rapidly; the volume of distribution is greater; and the fragment is eliminated with a far shorter half-life.¹⁰ In addition, when injected intravenously, Fab is less immunogenic than IgG.¹⁰ The immune complexes that may be formed are smaller than those that cause nephrotoxicity (comprising a single antigen molecule with several Fab attached), and complement cannot be fixed because the relevant binding sites on the Fc have been lost.

Renin-specific Fab has been used to block the action of renin in both sodium-depleted unanaesthetised dogs as well as in uninephrectomised dogs made hypertensive by constriction of the renal artery. In

both models, intravenous injection of Fab results in a hypotensive response (Fig. 1). When compared with intact antibody, the initiation of hypotension by Fab is more rapid, and the duration very much shorter. Repetitive experiments have been possible in the same animal without the problem of anaphylaxis that had been experienced previously when intact antibody had been used.

An antiserum is a mixture of several hundreds of antibodies, all capable of binding to the immunising antigen. These antibodies vary considerably both with respect to affinity for the antigen and specificity of recognition. The mixture of antibodies contained within an antiserum is different among even inbred (genetically identical) individual animals, as it is in the sera of a single animal collected at varying times after immunisation. Supplies of antisera are necessarily limited. Köhler and Milstein⁹ showed that antibodies might be produced in a very different manner. The technique of somatic-cell fusion allows for the production of hybrid cells. If the precursors of the hybrid are normal lymphocytes and cells from a malignant plasmacytoma, one can incorporate both properties of antibody production and growth *in vitro* into the product (colloquially named "hybridoma"). Cells that grow in culture may be cloned, so that all the progeny are daughters of a single precursor cell. A homogeneous culture of this type produces a single antibody that is uniform in structure and antigen binding properties. Since the cultures may be stored indefinitely at low temperatures, the same antibody may always be recovered. The amount of renin-specific antibody

available from conventional sera was severely limited; we set out, therefore, to make monoclonal renin-specific antibodies by the somatic-cell fusion method,²² making production in industrial quantities possible. Fig. 2 shows the binding to immobilised renin by a monoclonal antibody. We look forward to using monoclonal antibodies as more specific reagents that are available in indefinite quantities in the dissection of the physiology and chemistry of the renin-angiotensin system.

Blockade of beta-adrenergic receptor

The hormone receptors of the cell's plasma membrane are present in a very small number of copies (10 000 to 50 000 per cell), which makes their isolation exceedingly difficult. Antibodies to partially purified receptor preparations have been elicited,²³ but their utility is limited because they probably contain antibodies to other membrane constituents. The hormones or drugs that bind to receptors, however, are usually readily available in quantity. Frequently they are either peptides of modest size or synthetic organic compounds. For some receptors, several antagonists have been synthesised.

Could an antibody for the receptor be obtained by using the ligand (agonist or antagonist) as a template? The vast diversity of antibody combining sites, as already suggested, provides the potential for creating a complementary fit to almost any shape. If a figurative plaster mould could be cast upon the surface of the ligand that bound to the receptor, then a second

Fig. 1 Renin-specific Fab was administered intravenously to either uninephrectomised dogs that were hypertensive as a result of constriction of the renal artery (left) or sodium-depleted dogs (right). In both experiments the rate of fall in blood pressure is more rapid and the duration shorter than observed with intact antibody.

Fig. 2 Competitive radioimmunoassay of antibody 2B3-1A6. Renin is bound to the plastic microtitre plate and then incubated with the monoclonal antibody solution. The ordinate represents binding of ^{125}I goat anti-mouse Fab to IgM monoclonal antibody 2B3-1A6. Varying concentrations of soluble renin (abscissa) are added to the antibody solution before its application to immobilised renin. (Reprinted with permission of the American Heart Association, Inc. Hypertension, in press, 1981.)

mould made from the first one should have a perfect fit to the receptor. The well-known immunological principle of raising antibodies specific for another antibody's combining site (anti-idiotypic antibodies) may be used as the vehicle for moulding the desired shape. Certain refinements are needed to achieve the desired end. Only some of the atoms of either a hormone or an antagonist bond to the receptor. The rest of the structure is needed to stabilise the active site or possibly to protect it from degradation. To achieve the desired result, the first antibody must bind the ligand generally in the same way as the receptor does, and thus the same atoms and interatomic interactions must be used. There are two ways of achieving this end short of depending on fortuitous probability.

When conventional immunisation is employed, many antibodies to the immunogen are formed, each binding to it in a somewhat different manner. Some are relevant to the part of the ligand that binds to the receptor, while others are not. The antibodies of this polyclonal response may be fractionated by the use of ligands of different structure. By virtue of being receptor ligands, they are all capable of binding to the receptor and must have some common structures. If appropriately selected, structures irrelevant to receptor binding are not shared. Those components of the polyclonal antibody mixture that bind to a number of dissimilar ligands are likely to be similar to the recep-

tor. The most practical way of achieving fractionation with polyclonal antibodies is sequential affinity chromatography, a technique that employs a variety of ligands to capture the antibodies that bind to them. This general approach has now been applied successfully to the insulin receptor²⁴ and in our own laboratory to the beta-adrenergic receptor.²⁵⁻²⁷

Fig. 3 Competitive inhibition of $(-)-[^3\text{H}]$ dihydroalprenolol binding to unfractionated anti-alprenolol antiserum. Rabbit immune serum (100 μl) was diluted 1:10 in buffer and incubated for one hour at 25°C with $(-)-[^3\text{H}]$ dihydroalprenolol and increasing concentrations of $(\pm)$ -propranolol (Δ), $(\pm)$ -alprenolol ($\square$), $(\pm)$ -hydroxybenzylpindolol ($\bullet$), $(\pm)$ -norepinephrine ($\circ$), $(\pm)$ -isoprenaline ($\blacktriangle$), or catechol (∇). Each determination was performed in triplicate and corrected for the nonspecific $(-)-[^3\text{H}]$ dihydroalprenolol binding to rabbit preimmune serum. The results are expressed as a percentage of the total antibody-binding capacity for the tritiated antigen. (Reprinted with permission of the American Heart Association, Inc. Circ Res 1980; 46: 808-13.)

There is a wide variety of structurally different beta-antagonists available. All, however, share a common structure, a propanolamine side chain. Rabbits were immunised with the beta-antagonist alprenolol conjugated to a protein. The resulting antiserum was passed over an affinity column to which another beta-antagonist had been linked and all that was not bound was discarded. The column was then washed with 1-propranolol and the eluted antibody retained. Fig. 3 shows the specificity profile of this antibody fraction. Several beta-adrenergic antagonists as well as agonists are bound with considerable affinity, in some cases similar to that of the receptor. This fraction also resolved 1 and d stereoisomers of isoprenaline (not shown). Thus the antibody fraction could be viewed as a qualitative, but not a strictly precise quantitative, model for the beta-adrenergic receptor.

Table Inhibition of [^3H]alprenolol binding to antibodies and receptors by anti-idiotypic antibody*

	Maximal binding with preimmune (DPM)	In presence of anti-idiotypic (DPM)	Inhibition (%)
Original idiotypic (alprenolol)	10853 $\pm$ 250	488 $\pm$ 250	96%
Turkey erythrocyte	19643 $\pm$ 540	6195 $\pm$ 525	68%
Canine lung	6385 $\pm$ 283	4315 $\pm$ 190	34%

*Specific binding at 4-6 nM [^3H]alprenolol.(Reprinted with permission of Williams and Wilkins Co., Baltimore. *Pharm Rev*, in press, 1981.)

Antibodies specific for the antigen binding regions (complementarity regions) of the first antibody set were then raised by immunisation of rabbits that were genetically identical with respect to immunoglobulin constant region structures. Because of tolerance to self-determinants, the immunised animals made antibodies only to the variable regions, the unique structures on the immunogen. The antigenic determinant of an immunoglobulin that is characteristic of a single antibody species is called an idiotypic, and antibodies directed towards that determinant are named anti-idiotypes. The Table shows the inhibition of binding of a labelled beta-adrenergic antagonist, [^3H]alprenolol, to several kinds of binding sites by the anti-idiotypic antibody. Binding of the ligand, alprenolol, to the idiotypic (the first generation antibody that had been raised in response to alprenolol) is largely inhibited by anti-idiotypic (second-generation antibody). Of greater interest is the inhibition of the specific binding of alprenolol to plasma membrane preparation in two different animal species. Quantitative analysis of binding shows it to be of a competitive nature, analogous to that shown by conventional beta-antagonists.

The anti-idiotypic is also an inhibitor of adenylate cyclase activation by beta-adrenergic agonists. Increasing concentrations of isoprenaline progressively inhibit adenylate cyclase production in turkey erythrocyte membranes at $5 \times 10^{-7}\text{M}$ isoprenaline concentration. At a higher concentration of isoprenaline (10^{-4}M), less inhibition is observed, suggesting a competitive nature for this interaction as well. Thus the anti-idiotypic behaves as a true beta-adrenergic antagonist, competing with both agonists and antagonists for the receptor site. The obvious potential uses of such receptor-specific antibodies are: the recognition of structural differences among subsets of beta-adrenergic receptors²⁸; a more rigorous examination of their respective physiological role using reagents of greater resolution; and the isolation of receptors with antibody affinity chromatography.

Imaging of myocardial infarcts using cardiac myosin-specific antibodies

The hallmark of cell death is loss of the membrane integrity: when there is no longer a physical separation between inside and outside, the cell ceases to exist. This principle is commonly employed in clinical practice in diagnosing tissue infarction. Intracellular enzymes are lost to extracellular fluid, and their increased concentration in the blood may be used as a measure of tissue necrosis. An alternative approach is the inward diffusion of a marker that is normally excluded from cells. We have selected myocardial infarction as a model and radioactively labelled myosin-specific antibody as a marker.

Myosin is the principal protein of the cardiac cell. Its covalent structure is unique to the heart,²⁹ allowing the development of antibodies that differentiate between cardiac and either skeletal- or smooth-muscle myosins. In the intact organism, cardiac myosin is protected from extracellular fluid by the cell's plasma membrane. When cell death occurs and the membrane breaks down, myosin is exposed to extracellular fluid. It is then available to react with labelled antibodies or antibody fragments.

A visual demonstration of this phenomenon is seen in Fig. 4. The figure shows a scanning electron micrograph of part of a neonatal mouse cardiac myosite that had been rendered ischaemic by prolonged exposure to nitrogen and then incubated with $1\ \mu$ diameter, polystyrene spheres covalently bound to myosin-specific antibody. Myofibrils are seen protruding from a hole in the cell membrane. Antibody-coated microspheres clearly bind to the myofibrils. Not only is myosin very insoluble in physiological fluids so that membrane breakdown does not result in antigen loss, but it persists for considerable periods after ischaemic necrosis.³⁰ This should allow for identification of infarcted myocardium in days to, perhaps, several weeks after the initial event. In our initial exploration of this concept, a canine myocardial infarction model was used.³¹ The left anterior descending coronary artery was ligated, and four hours later ^{125}I -labelled, myosin-specific antibody or antibody fragments were injected intravenously. At varying times after the injections, the animals were killed, the hearts were perfused with triphenyltetrazolium chloride, and the myocardia examined.³² Fig. 5 (left) shows a section of a heart treated in this way. The light area represents a largely subendocardial infarction. The central panel of the figure is a tracing of the slice superimposed on an autoradiograph. The exposed area corresponds to the infarct as disclosed by the triphenyltetrazolium stain. The right panel shows specific radioactivity in the area of the infarct relative to myocardium on the posterior wall. It is apparent that the major concentra-

Fig. 4 Scanning electron micrograph of an ischaemic neonatal mouse myosite (magnification $\times 2400$). The spheres adherent to the cell are covalently bonded to myosin-specific antibody. Non-ischaemic cells do not bind significant numbers of spheres.

Fig. 5 Histochemically delineated myocardial infarction in a ventricular slide (left) seen as white or lighter-coloured regions, and normal myocardium seen as darker regions. The corresponding macroautoradiograph is shown in the centre with the outline of the ventricular slide. The right panel shows relative antibody uptake in the indicated areas. Ratios of antibody uptake were determined as specific radioactivity in relation to normal posterior ventricular myocardium. (Reprinted with permission of the American Heart Association, Inc. Proc N Engl Cardiovasc Soc 1979: Vol. II; 31–8.)

tion of radioactivity is in the subendocardial region, with lesser concentration in the subepicardial region that had been subject only to spotty necrosis as indicated in the triphenyltetrazolium stain.

Microautoradiographs showed that individual necrotic myosites could be identified and differentiated from adjacent viable cells.³³ In order to show that antibody concentration was specific and not simply the result of passive diffusion of a macromolecule into infarcted cells, specific antibody labelled with ^{131}I and nonimmune globulin labelled with ^{125}I were injected

simultaneously into the coronary arteries.³² At the centre of the infarct, the antibody has concentrated 34-fold in relation to normal myocardium, while the nonimmune globulin is only sevenfold in excess. In normal tissue, as expected, concentrations are equal. When compared to a marker of relative flow, the distribution of radioactive microspheres which had been injected into the left atrium, it was clear that the concentration of labelled antibody was inversely related to relative blood flow.^{31 32} There seems to be sufficient collateral circulation in this ischaemic model

to provide for delivery of antibody, even to the regions of minimal blood flow. It would be very desirable to apply this method to the detection, localisation, and quantification of myocardial infarcts in the living subject.

Optimal imaging with a gamma or positron camera requires the labelling of antibodies with radionuclides of appropriate half-life and affinity. We have accomplished this by covalently linking diethylene triamine penta-acetic acid (DTPA) to antibody or antibody fragment and then binding cationic radionuclides by chelation.³⁴ Successful images have been obtained using the following radionuclides linked to antibody: ¹¹¹In-DTPA-Ab Fab,³⁴ ⁶⁸Ga-DTPA Fab,³⁵ and ^{99m}Tc-DTPA-Ab Fab.³⁴ An example of a positron image of an anterior infarct in a living dog using ⁶⁸Ga-labelled antibody is shown in Fig. 6 (left). The positron imaging technique allows for tomographic reconstruction and, thus, the heart is visible on cross-section in the ¹³N-ammonia image in the centre panel of Fig. 6 (ammonium ion is a potassium analogue and concentrates in normal tissue). On the left side of the image there is clearly less density, reflecting the region of infarction. Superimposition of the ¹³N and the ⁶⁸Ga images (right panel) showed that the antibody was concentrated directly in the area of diminished ammonium ion uptake. The potential for applying this method to the evaluation of myocardial infarcts in a clinical setting seems very great indeed since it offers high specificity and resolution.

Reversal of digitalis glycoside intoxication

If the effect of an endogenously produced hormone

can be reversed, it should also be possible to counteract the undesirable effects of an exogenously administered drug or toxin. The digitalis glycosides are of great value in the treatment of congestive heart failure and consequently are frequently used in clinical medicine. Unfortunately, they are characterised by a very close toxic-therapeutic ratio. Digitalis intoxication is one of the most frequent adverse drug reactions reported. There is no specific antidote, and the cardiac arrhythmias that are a feature of digitalis intoxication are not uncommonly fatal.

We reasoned that if an antibody specific to the digitalis glycosides had a higher affinity for the drug than the physiological receptor, it should be possible to transfer the ligand from the receptor to antibody, simply by mass action. For optimal effectiveness diffusion distances should be minimal, and the antibody should be in high concentration in extracellular fluid in proximity to the receptor. It would also be desirable to remove the antibody-drug complex rapidly from the body. Both aims may be accomplished by the use of digitalis-specific Fab. As indicated above, the volume of distribution of Fab is greater than intact antibody since it readily enters extracellular fluid. It is also of sufficiently small size (50 000 Daltons) to pass through the glomerular filter and be largely excreted in the urine.³⁶

Digoxin-specific antibody was purified from sheep antiserum using immobilised ouabain Fab isolated after papain cleavage.³⁷ After demonstration of safety and effectiveness in animal studies, clinical investigations were initiated. At the time of this writing, 17 patients with life-threatening digitalis intoxication have been studied. The patients ranged in age from

Fig. 6 ⁶⁸Ga and ¹³NH₄⁺ were obtained sequentially using a positron camera; 5 mCi of ¹³NH₄⁺ was administered intravenously to a dog that had had occlusion of the distal segment of the left anterior descending artery for four hours and was imaged (centre); 30 minutes later 1 mCi of ⁶⁸Ga was injected into the same artery and imaged (left panel). Right panel is a composite of both images. (Reprinted with permission of the American Heart Association, Inc. Proc N Engl Cardiovasc Soc 1979: Vol. II; 31-8.)

infants to septuagenarians and in each case a dramatic reversal of the signs and symptoms of intoxication occurred.³⁸⁻⁴⁰

The history of a recently reported patient is typical of the group.³⁹ She was a 34-year-old wife of a physician, who, because of marital difficulties, took 20 mg digitoxin, a massive overdose. She appeared well on admission to the hospital except for nausea, but soon lapsed into a series of life-threatening arrhythmias that included multiple episodes of ventricular fibrillation, which were treated with countershock, as well as asystole, which was treated by ventricular pacing. At the time the antibody Fab became available to the physicians treating her, the patient was in shock and anuric, and she had dilated pupils. Her serum potassium was raised, a grave prognostic sign in digitalis intoxication.⁴¹ Within an hour after the intravenous administration of antibody Fab, her atrioventricular conduction had returned, and she was soon in normal sinus rhythm. No further arrhythmias occurred. She was discharged from hospital without sequelae several days later. Fig. 7 shows the initial distinct increase of serum digitoxin concentration in this patient as

Fig. 7 Blood levels of digitoxin and Fab after intravenous administration of antibody Fab to a 34-year-old woman suffering a series of life-threatening arrhythmias as a result of a massive overdose (20 mg) of digitoxin. Within an hour of intravenous administration of antibody Fab, atrioventricular conduction had returned. (Reprinted from Contributions of chemical biology to the biomedical sciences with permission of Academic Press, New York. In press, 1981.)

tissue-bound drug equilibrated with the antibody (antibody-bound drug is pharmacologically inactive), followed by rapid clearance of both drug and Fab. It should be noted that the half-life of digitoxin in man is normally three and a half days when hepatic metabolism of the drug is the major source of removal. It is

apparent that excretion was much accelerated by the antibody Fab, the half-life apparently reduced to about 12 hours. Fig. 8 shows that both antibody and Fab appear in the urine in largest amounts within the first day after Fab administration.

Fig. 8 Urinary excretion rates of digitoxin and Fab after intravenous administration of antibody Fab to a 34-year-old woman suffering from digitalis intoxication (20 mg). (Reprinted from Contributions of chemical biology to the biomedical sciences with permission of Academic Press, New York. In press, 1981.)

The digitalis glycosides in most common use are digoxin and digitoxin. They differ from one another very little in their chemical structure, yet most antibodies differentiate between them, with differences in affinity of 50- to 100-fold.² It would be desirable to use an antibody that bound both digoxin and digitoxin with equal affinity. A monoclonal antibody (designated 26-10 in our laboratory) was selected that had an affinity of 5×10^9 for digoxin and a nearly equal affinity for digitoxin.⁴² Since a monoclonal antibody is a homogeneous protein that recognises only a single aspect of an antigen molecule, it appears that the hydroxyl group is not a part of the antigenic recognition site of this molecule. This antibody, which can be produced in quantities without limit, has been shown to reverse digoxin intoxication in experimental animals.

It should be apparent that this approach could be applied to many other drugs or toxins. Of particular interest is the acceleration of excretion of a substance that is normally metabolised slowly. Fab is capable of altering the route of disposal from hepatic metabolism to renal excretion.⁴³

Antibodies as drugs

The development of a new drug is often the result of empiricism, serendipity, or the deliberate modification of an existing natural product. These products of the organic chemist's laboratory are usually characterised by a lack of specificity with consequent abundance of adverse reactions. If antibodies were the basis of identification of a specific site within the body for binding, blockade, or modification, one could choose among some 10^{11} different recognition sites and thus much increase specificity. Adverse reactions to drugs are most often the result of reaction with a site other than the desired target. Selective interaction should, of necessity, diminish these undesired effects. The considerably larger size of the antibody combining site in comparison to conventional drugs allows for the establishment of a greater number of specific interactions among atoms and thus both greater affinity and specificity.

While some of the advantages of antibodies are obvious, the disadvantages, discussed in part above, must also be considered. When binding to a specific site is all that is desired, the activation of the biological effects that are an intrinsic part of an immune reaction is undesirable. The smaller Fab that was discussed above as a possible solution to some of these problems still carries with it an entire domain (one half of its mass) that is of no relevance to antigen binding. Fab from a heterologous species, while of diminished immunogenicity, is likely to cause hypersensitivity when used on a long-term basis. The solutions to these problems are almost at hand. A much smaller fragment of antibody has been produced that retains all the binding energy and specificity of the intact molecule. Fv comprises a single domain and has a molecular size of 25 000 Daltons.⁴⁴ While its pharmacokinetics have not as yet been examined, I anticipate that it will be very rapidly cleared and distributed. The advent of the hybridoma method will make possible not only the selection and large-scale production of antibodies of uniform properties, but also the reduction or elimination of the potential problem of hypersensitivity, since it will be possible, as indicated above, to produce human antibodies. Will the formation of anti-idiotypic antibodies defeat the long-term utility of antibody therapy? I believe that the well-recognised difficulty in producing anti-idiotypes, even deliberately, indicates that this problem will be rare. The next decade will see a new pharmacology based on the antibody combining site.

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