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Immunotherapy in the Poisoned Patient
Overview of Present Applications and Future Trends

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Summary

Immunotherapy for reversal of toxicity due to poisons and drugs is not new. However, refinements in antibody isolation and purification as well as the advancement of hybridoma technology and recombinant DNA biotechnology has led to a new generation of immunotherapeutic and diagnostic agents.

The advent of monoclonal antibody technology in 1975 heralded the new age of immunopharmacology and immunotoxicology. Monoclonal antibodies designed for a specific antigen resolved the problem of polyclonality and cross-reactivity of traditional antibodies. Along with the production and isolation of active antibody fragments from both polyclonal and human monoclonal sources, as well as the ability to tailor-make chimeric antibodies by recombinant biotechnology, the development of novel immunotherapeutic agents has taken place. Two immunotherapeutic modalities, digoxin-specific antibody fragments (Fab) and snake antivenin, have been available for the clinician's armamentarium for years. Along the same lines of anti-digoxin Fab development, application of newer antibody isolation technology has led to a purified IgG(T) antibody for snake venom poisoning which is still in the developmental stages.

Potential future developments in immunotherapeutics must overcome the clinical problems of immunogenicity and adverse reactions to the antibodies. Human monoclonal sources, active antibody fragments, and chimeric antibodies from transfectomas are all potential resolutions to these problems.

The concept and practice of using antibodies for therapy in specific human intoxications is an historically accepted management modality. Disease produced by Clostridial organisms, *C. tetani* and *C. botulinum*, as well as *Corynebacterium diphtheriae*, were treated in the nineteenth century with antisera derived from toxins elaborated by these microbes. The use of antivenins for neutralisation of the protein poisons from snakes, scorpions, and spiders has been an accepted practice for more than 30 years. Historically, antibody neutralisation of toxins has been directed mainly at protein poisons from animals, microbes, plants, and insects.

The advent of more sophisticated immunisation, haptenisation, and antisera purification techniques has allowed development of antibodies to low molecular weight drugs. To elicit the production of specific antibodies to low molecular weight substances, these drugs must be coupled to a larger protein carrier, such as albumin, by commonly em-

ployed haptenisation processes. The drug may be coupled directly to the protein via a primary or secondary amino group or it may have to be derivitised to form a more favourable reactive coupling group (Avrameas et al. 1978; Erlanger 1973). The protein carrier molecule is obtained from a different animal source than the animal being immunised. When immunised at appropriate intervals, the host will develop antibodies against both the hapten (drug) and the protein carrier. The production of the antibodies to small molecules has been extensively reviewed by Butler and Beiser (1973).

Antibodies are currently being used to reverse toxicity from drugs and toxins, as drug carriers in cancer therapy, as imaging agents, and in immunoassays (table I). The availability of purified antibodies has also led to development of sensitive new immunoassays for the detection of drugs and toxins (Butler 1977). These immunoassays have traditionally been radioimmunoassays, but now less expensive enzyme immunoassays are being employed clinically to detect a wide variety of drugs and toxins.

Antibodies developed to a drug inhibit the pharmacological activity of that drug *in vivo* and *in vitro* and are thus capable of blocking the toxicological effect. Early experience in developing immunoassays led to investigations in the 1970s which demonstrated the effectiveness of anti-digoxin antibodies in reversing digitalis toxicity. These studies resulted in the clinical concept of potential immunotherapy for other drugs and toxins.

Table I. Some small molecules to which antibodies have been made

Amanitin	Amikacin
Amphetamine	Amitriptyline
Aspirin	Arsenicals
Atropine	Barbiturates
Cardiac glycosides	Caffeine
Carbamazepine	Carcinogens
Chlordiazepoxide	Chlorpromazine
Chloramphenicol	Clomipramine
Clindamycin	Clonidine
Cocaine	Codeine
Colchicine	Corticosteroids
Desipramine	Diazepam
2,5-Dimethoxy-4-methylamphetamine	Ethosuximide
	Glutethimide
Gentamicin	Hydralazine
Haloperidol	Indomethacin
Hydromorphone	Isoniazid
Insecticides	Mescaline
Lysergic acid diethylamide (LSD)	Methaqualone
Methadone	Naloxone
Morphine	Paralytic shellfish poison
Nortriptyline	Pentazocine
Paraquat	Pethidine (meperidine)
Penicillin	Phenylbutazone
Phenobarbitone	Procainamide
Phenytoin	Quinidine
Primidone	Tartrazine
Propranolol	Theophylline
Strychnine	Warfarin
Tetrahydrocannabinol (THC)	
Tobramycin	

1. Antibodies as Physiological Antagonists of Drugs and Toxins

1.1 Structure and Function of Antibody Molecules

Antibodies have become new pharmacological and diagnostic agents in medicine. The basic structure of the antibody molecule (fig. 1) was described in 1959 (Porter 1959) and investigations since then have further delineated antibody structure and function through enzymatic cleavage of the whole antibody into active subcomponents (Nisonoff 1964). Functionally, antibodies are able to bind to one or many different antigens, as well as carry out effector roles involving complement binding and activation, stimulation of macrophage activity and phagocytosis, and release of vasoactive substances from granulocytes. Antibody binding is determined by the protein structure of the variable regions of the light and heavy chains (V_L and V_H). The effector function of the antibody is contained in the Fc region, or constant region; this region has relatively few functions compared with the variable region. The variable, or affinity region, binds to a wide variety of antigens. Estimates are that over 10^{10} different antibody combinations exist (Haber 1982).

When an antibody is produced by an animal, the antibody formed in response to a specific antigen may bind to several sites on that antigen; many different antibodies are formed within an antibody class to carry out this binding. This is referred to as the polyclonality and polyvalency of antibodies.

Human antibodies have 8 possible constant regions. Each constant region represents a different antibody class. Conventional immunisation techniques result in polyclonal, polyvalent antibodies. Since most antigen preparations are impure, the resulting antiserum contains a wide variety of antibodies directed against many different antigenic sites of the immunising mixture.

1.1.1 Antibody Fragments

When digested by pepsin, the typical IgG molecule yields 2 fragments: an Fab_2 and the Fc. The Fab_2 fragment has a molecular weight of approximately 100,000. Enzymatic cleavage with papain results in 3 fragments: 2 Fabs with molecular weights of 50,000; and the Fc region, with a molecular weight of approximately 80,000 (Nisonoff 1964). The Fab_2 and the Fab fragments contain the high-affinity active antibody combining sites. Further digestion of the IgG antibody yields an even smaller active fragment containing the terminal hypervariable Fv region. The Fv region is composed of a heavy and light chain component with

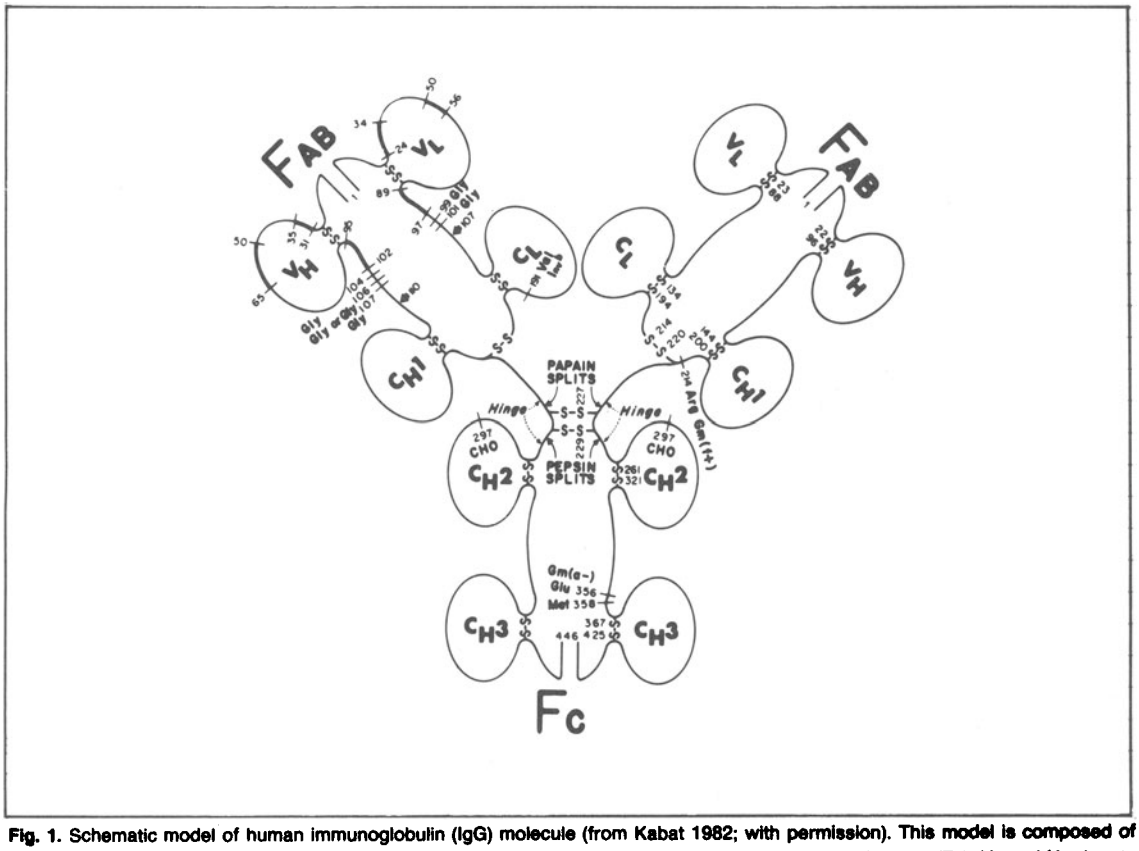

Fig. 1. Schematic model of human immunoglobulin (IgG) molecule (from Kabat 1982; with permission). This model is composed of 2 heavy and 2 light polypeptide chains and has 2 antigen binding sites (Fab) and a complement fixing site (Fc). V_L and V_H denote light- and heavy-chain variable regions, respectively, and C_H1,C_H2, and C_H3 are domains of constant region of heavy chain; C_L is constant region of light chain.

a molecular weight of 25,000 and is the smallest active antibody fragment (Hochman et al. 1973). The actual combining site regions of the heavy and light chains contacting the antigen are only 12,000 Daltons each (Haber 1983).

1.1.2 Monoclonal Antibodies

Antibodies possess some ideal features that make them useful for pharmacological application as drugs: high specificity, high degree of antigen resolution, large number of possible combinations, ease in production, and utility of active fragments. These features have been reviewed in detail (Butler 1982; Haber 1982).

There are, however, problems associated with the use of antibodies as therapeutic and diagnostic agents, most of which are related to the heterogeneity and polyclonality of immunoglobulins. These problems were overcome in 1975 when Kohler and Milstein demonstrated that antibodies of a single designed specificity could be produced from a hybrid of fused mouse myeloma cells with normal mouse spleen cells. The resulting 'hybridoma' produced monoclonal antibodies in large quantities specific for a single antigenic determinant (Kohler & Milstein 1975). Since the advent of monoclonal antibodies, many uses have been found in diagnostics as well as potential therapy for some forms of cancer. Since each hybridoma produces antibodies with identical variable regions, hybridoma technology rapidly became a source of homogenous antibodies.

1.2 Pharmacokinetic Characteristics of Antibodies and their Fragments and Mechanisms of Toxicity Reversal

The pharmacokinetic characteristics of sheep anti-digoxin IgG and their Fab fragments have been investigated by Smith et al. (1979). As with other drugs, the pharmacokinetics of antibodies can be described in terms of their volume of distribution, elimination half-life, and plasma clearance (table II). However, IgG from a foreign source, no matter how pure, is immunogenic. Also, because of its large molecular weight, IgG is cleared by cells of the immune system, thus setting up the potential for serum sickness (antigen excess) reactions. Fab fragments of IgG, which are much smaller in size and readily eliminated renally are much less immunogenic than the whole IgG. Since Fab fragments have the same affinity for antigen as IgG, they are better suited for toxin and drug neutralisation as well as for enhancing their elimination.

Table II. Some pharmacokinetic parameters of IgG and Fab fragments (after Smith et al. 1979)

Parameter	IgG	Fab fragments
Vd (L/kg)	0.053	0.462
$t_{½\alpha}$ (h)	4	0.28
$t_{½}$ (h)	61	9.1
CL (ml/min/kg)	0.009	0.56

Abbreviations: Vd = volume of distribution; $t_{½\alpha}$ = distribution half-life; $t_{½}$ = elimination half-life; CL = total body clearance.

In reversing drug-induced toxicity, IgG or Fab fragments are usually administered intravenously. IgG has a very small volume of distribution compared with Fab fragments and does not penetrate fully into the body water. Fab fragments, on the other hand, have a relatively large volume of distribution but do not distribute to receptor sites of drugs with large volumes of distribution. Therefore, other mechanisms of toxicity reversal must occur away from the tissue receptor level of a drug with high tissue binding such as digoxin. Probable mechanisms have been suggested (Butler 1982; Skubitz & Smith 1975) and include the following:

1. Binding drug in the extracellular fluid
2. Creating a concentration gradient to the central compartment
3. Binding recently dissociated drug.

All of these mechanisms probably have some validity.

Alteration of drug kinetics by specific IgG and Fab fragments is the goal of immunotherapy. Such alteration includes enhancing drug elimination. It

is thought that the micro rate constant for a drug or toxin is altered in favour of redistribution to the central compartment from peripheral compartments. This may only be true for a drug or toxin whose distribution (k_{12}) and redistribution (k_{21}) rate constants are in equilibrium. If the distribution rate constant is extremely large, as it would be for a toxin like polychlorinated biphenyls (Matthews 1984), then immunotherapy would not tend to favour redistribution, binding, and elimination of that toxin.

Binding of drug by antibody is a reversible event. Again, the use of rapidly eliminated Fab fragments would serve to help eliminate more drug than the slowly eliminated IgG. Increased binding of drug in the extracellular space and central compartment will decrease the amount of drug available to interact with tissue receptor sites and thus reduce toxicity.

2. Clinical Considerations and Complications of Immunotherapy

2.1 Immunogenicity and Cross-Reactivity with Other Drugs

Despite the obvious advantages of antibodies over traditional drugs, there are serious problems yet to be overcome. One may wonder why antibody therapy is not available for treating virtually every drug in overdose and toxicity. Immunoglobulins, unless obtained from a human source, are immunogenic. The use of antibody fragments has helped to diminish the problem of immunogenicity to a great extent but even the Fab fragments from a heterologous source are immunogenic (Smith et al. 1979). If a patient requires treatment with an immunotherapeutic agent more than once, he or she may experience an acute IgE-mediated hypersensitivity reaction following repeated administration of the same antibody. The polyclonal nature of traditionally derived antibodies results in cross-reactivity with other drugs and may, therefore, decrease the efficacy of this form of treatment in a patient with polydrug overdose involving drugs with similar chemical structures such as desipramine and carbamazepine.

2.2 Dosage Considerations

Some drugs are simply not amenable to neutralisation by antibodies. This may be especially true when a drug is ingested in gram amounts. Since one mole of Fab will combine with one mole of drug, 10mg of a toxin with a molecular weight of 300 is stoichiometrically equivalent to about one gram of purified Fab fragments. Thus overdose with a drug taken in gram quantities would require huge amounts of Fab fragments or whole IgG for total body neutralisation. However, it is possible that if the kinetics of the 'toxic compartment' can be favourably altered with relatively low doses of Fab fragments, despite leaving the remainder of the drug unbound, then total body burden of drug need not be neutralised to favourably alter a state of intoxication. Current investigations are ongoing with tricyclic antidepressants in this regard (Pentel et al. 1985).

If there is a large body-burden and slow redistribution of drug from tissue, Fab fragments may be eliminated more rapidly than the offending drug. In this case, the drug may re-equilibrate and reproduce toxicity thus requiring continuous infusion or intermittent administration of the antibody fragment, creating the potential for serum sickness reactions.

2.3 Hypersensitivity Reactions with Antivenin Immunotherapy

The majority of clinical complications associated with antivenin immunotherapy are related to acute hypersensitivity reactions and serum sickness reactions.

2.3.1 Anaphylactic and Anaphylactoid Reactions

Acute reactions can be subdivided into 2 groups: anaphylactic and anaphylactoid. Anaphylaxis is mediated via IgE whereas anaphylactoid reactions are non-IgE-mediated (Foreman 1981; James & Austen 1964; Kelly & Patterson 1974; Sheffer 1984; Zweiman et al. 1975). The clinical syndrome is identical in both anaphylaxis and anaphylactoid

reactions and is clinically managed the same way.

The administration of large amounts of foreign protein intravenously is thought to activate the complement system and in some circumstances to result in an anaphylactoid reaction with signs and symptoms of hypotension, urticarial rash, and bronchospasm (Sheffer 1984; Sutherland 1977). Anaphylaxis presents as a spectrum from shortness of breath, urticarial rash, and bronchospasm to hypotension, angioedema, shock, and death. Early and aggressive treatment with intravenous crystalloids, subcutaneous adrenaline (epinephrine) and antihistamines can terminate progression of the syndrome and be life-saving. When treating anaphylaxis, a special situation exists in patients taking β-adrenoceptor blocking drugs such as propranolol; anaphylaxis in these patients can be usually severe and difficult to manage (Smith et al. 1980). Propranolol blocks adenyl cyclase receptors on efferent cells and results in lower intracellular concentrations of cyclic AMP thus reducing the threshold release of vasoactive substances from mast cells and basophils (Jacobs et al. 1981). β-adrenoceptor blockers also decrease the pharmacological response to adrenaline in both the cardiovascular and respiratory systems (Harris et al. 1966; Kaliner et al. 1972).

2.3.2 Serum Sickness Reactions

Serum sickness reactions are delayed and due to formation of immune complexes of antibody formed against the administration of excess foreign protein. Clinical signs and symptoms may appear anywhere from a week to a month following exposure to the foreign protein. Clinical manifestations of serum sickness include arthralgias, fever, urticarial rash, proteinuria, and lymphadenopathy. Recently, direct immunofluorescence of skin lesions has revealed the presence of immune deposits consisting of IgM, IgA, IgE, and complement (Lawley et al. 1984).

Treatment of serum sickness reactions has traditionally consisted of corticosteroid and antihistamine administration. However, in one clinical series, corticosteroids were of no demonstrable benefit (Lawley et al. 1984).

3. Current Clinical Applications of Immunotherapy

Two current clinical applications of immunotherapy in human poisoning involve reversal of severe digitalis poisoning and treatment of snake envenomation. The efficacy of antibody therapy in both of these situations is established.

3.1 Immunotherapy for Severe Digoxin and Digitoxin Poisoning

Although commonly ingested in acute overdose, digoxin and other cardiac glycosides are not generally associated with severe, life-threatening intoxication in the great majority of instances. However, when severe toxicity develops from acute overdose, treatment is mainly supportive and mortality is high. Life-threatening complications include ventricular fibrillation, ventricular tachycardia, hyperkalaemia, and severe atrioventricular block.

The development of an immunotherapeutic agent for digitalis intoxication is a practical application of antibody therapy for drug intoxication. Because digoxin is taken therapeutically in small amounts of 0.125 to 0.25mg, and because in acute overdose the amount ingested is usually only several milligrams, stoichimetrically it is feasible to neutralise digoxin with antibody on a mole for mole basis. Digoxin is the most commonly used cardiac glycoside and differs from digotoxin by a single hydroxyl group in position 12 on the steroid ring. Pharmacological activity resides in the aglycone portion of the molecule. Thus it was reasoned that an antibody developed to digoxin would also cross-react with digitoxin. This proved to be true.

In the early developmental stages of digoxin-specific antibodies, rabbits were immunised with digoxin-bovine serum albumin conjugates to develop antibodies to the haptenised digoxin molecule. The resulting antiserum demonstrated specific binding to digoxin as well as cross-reactivity to digitoxin (Butler & Chen 1967). Later, sheep digoxin-specific IgG was developed and isolated using affinity chromatography. Since heterologous

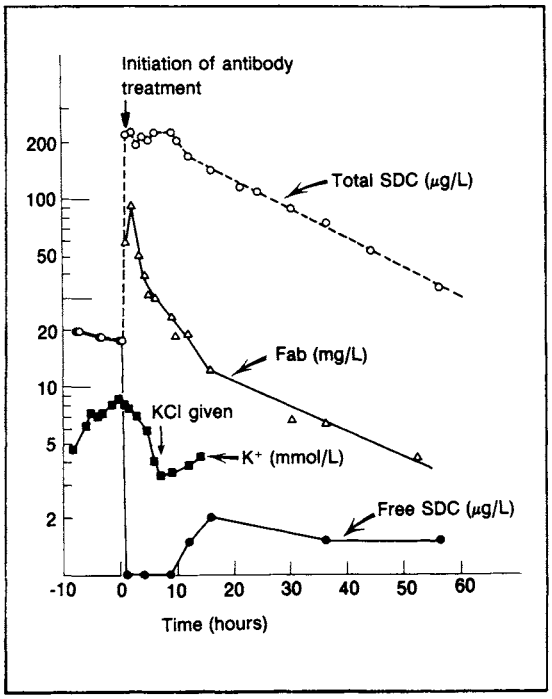

Fig. 2. Time course of total (O---O) and free (●——●) serum digoxin concentration (SDC); serum concentration of sheep digoxin-specific antibody fragments (Fab; Δ——Δ); and serum potassium concentration (K^+; ■—■) after intravenous administration of antibody to a 39-year-old man with severe digoxin poisoning (from Smith et al. 1976; with permission).

IgG maintains immunogenicity, Fab fragments were produced by papain digestion of the whole antibody followed by isolation of active fragments (Curd et al. 1971). The use of Fab fragments was more feasible for 3 principal reasons: (1) the pharmacokinetic characteristics of the Fab fragments are more favourable than the whole IgG; (2) Fab fragments are less immunogenic than IgG; and (3) Fab fragments are renally cleared and will enhance digoxin elimination.

The efficacy of anti-digoxin Fab fragments was initially established in animal studies in which ventricular tachycardia in dogs was reversed following intravenous infusion of the fragments (Curd et al. 1971). The first clinical report of the success of anti-digoxin Fab fragments was in 1976. The signs and symptoms of an acute overdose of approximately 22.5mg of digoxin (i.e. severe atrioventricular block and hyperkalaemia of 8.7 mmol/L) were reversed by use of Fab fragments (Smith et al. 1976). Pharmacokinetic data collected in this patient demonstrated a rapid fall in the free serum digoxin concentration together with a marked elevation in total (i.e. Fab bound) serum digoxin concentration (fig. 2); an increased urinary excretion of both digoxin and Fab fragments was also documented, with urinary total digoxin concentrations up to 960 μg/L recorded during the first day.

Further clinical investigations confirmed the efficacy of anti-digoxin Fab fragments in acute overdosage of both digoxin and digitoxin (Bismuth et al. 1982; Ochs & Smith 1977; Smith et al. 1982). Data collected in the case of a 34-year-old female with severe digitoxin intoxication revealed increased plasma concentrations of Fab-bound digitoxin as well as increased urinary excretion of both digitoxin and digoxin-specific antibody fragments (figs 3 and 4); her clinical condition was markedly improved within 1 hour of administering Fab. In another study, treatment with digoxin-specific

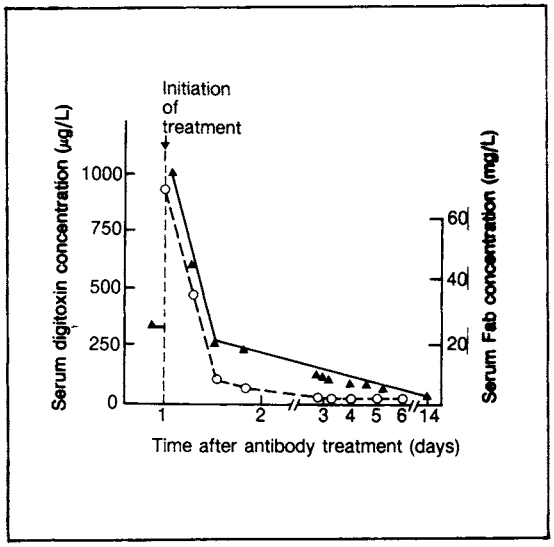

Fig. 3. Total serum digitoxin (▲—▲) and digoxin-specific antibody fragments (Fab; o---o) during and after intravenous antibody therapy in a 34-year-old woman with life-threatening arrhythmias due to massive digitoxin overdose (20mg) [reproduced with permission from Aeberhard et al. 1980].

antibody fragments rapidly reversed the signs and symptoms of accidental digitoxin poisoning in a 77-year-old man; this was associated with a dramatic rise in the platelet count within 8 to 16 hours of starting the antibody infusion (Hess et al. 1983) [fig. 5].

Criteria for treatment with Fab fragments include failure of conventional supportive care to reverse life-threatening dysrhythmias and hyperkalaemia.

3.2 Immunotherapy for Snake Venom Poisoning

Snake venom poisoning remains a worldwide concern and represents significant morbidity and mortality in some countries. Estimations are that 50,000 deaths per year occur globally from snake venom poisoning (Russell 1983). In developing agricultural countries, envenomations are an occupational hazard.

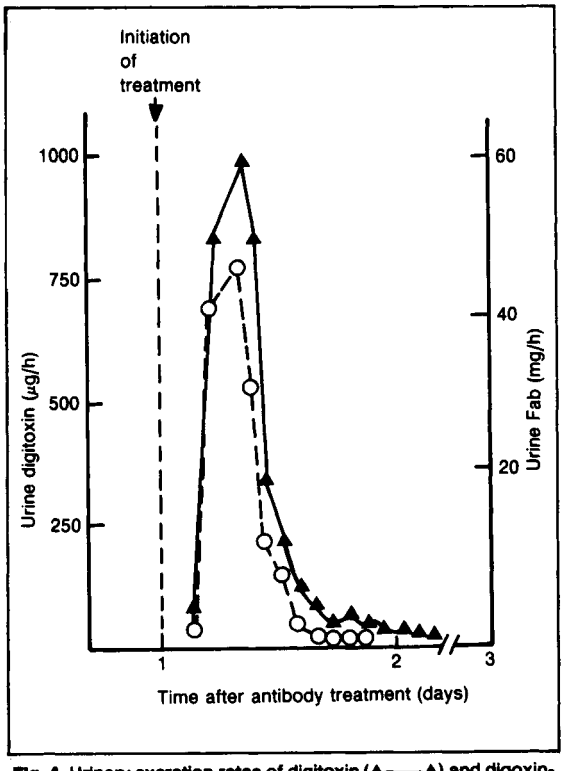

Fig. 4. Urinary excretion rates of digitoxin (▲——▲) and digoxin-specific antibody fragments (Fab; o—o) following intravenous administration of antibody to a 34-year-old woman suffering from massive digitoxin overdose (20mg) [reproduced with permission from Aeberhard et al. 1980].

3.2.1 Problems in the Use of Currently Available Snake Antivenins

Snake antivenins are produced by gradually immunising horses with venom antigens. This production and processing of antivenins has not changed in 30 years. All snake antivenins are equine in origin and contain extraneous proteins; thus there is a need for a commercially available purified antibody product.

Two main clinical problems relate to the therapeutic use of present day antivenins: (a) it is difficult to determine the amount of antivenin required; and (b) adverse reactions to antivenins are common. The commercial processing of antivenin by the 30 worldwide manufacturers varies from use of whole hyperimmune horse serum to ammonium sulphate precipitation of immunoglobulins from antisera, to enzymatic refinement of antisera (Latifi 1978; Russell 1983). The final product may be liquid or freeze-dried. The enzymatic/ammonium phosphate method has been employed commercially since 1939. Some refinements in antivenin production have occurred with the use of pepsin digestion of whole antisera by the Commonwealth Serum Laboratories in Australia.

Depending on the final product and the amount of antivenin administered, serum reactions vary from almost none to over 75%. These serum reactions are mainly antigen excess serum sickness, but acute IgE-mediated hypersensitivity reactions as well as anaphylactoid reactions also occur (Russell 1983).

Immunisation of horses with venom antigens stimulates the production of IgG antibodies to various venom proteins and peptides. As demonstrated by isoelectric focusing (fig. 6), snake venom contains well over 100 proteins and peptides whose toxicological activity is not yet known (Russell & Egen 1984).

Commercially available antivenin in the US is

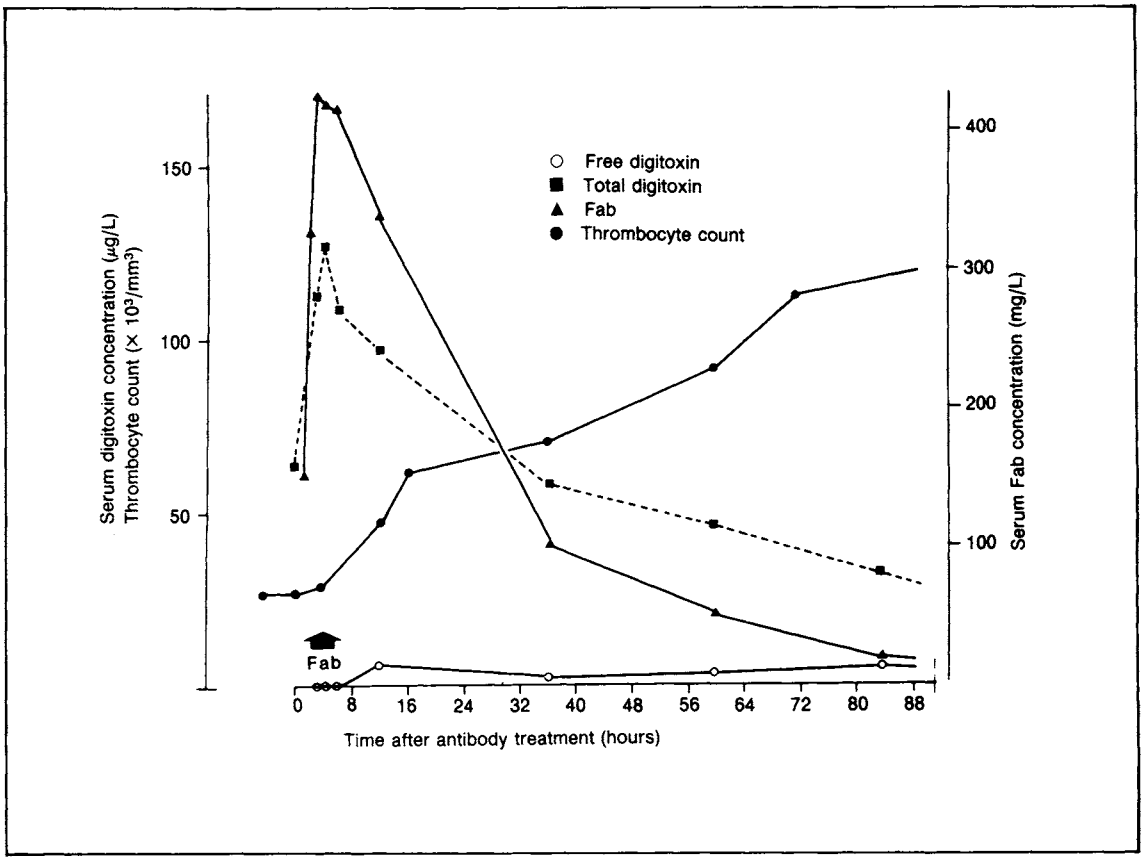

Fig. 5. Serum concentrations of free and total digitoxin; digoxin-specific antibody fragments (Fab); and the platelet count during and after antibody therapy in a 77-year-old man suffering from cardiac arrhythmias and severe thrombocytopenia after accidental digitoxin poisoning (from Hess et al. 1983).

produced by Wyeth Laboratories. This product is the result of immunising horses with 4 separate venoms from North American crotalids: *Crotalus atrox* (Western Diamondback rattlesnake), *Crotalus adamanteus* (Eastern Diamondback rattlesnake), *Bothrops atrox* (Fer-de-Lance), and *Crotalus durissus terrificus* (Tropical rattlesnake). The resulting antisera is processed by ammonium sulphate precipitation and the final sterile product is freeze-dried. This process method has been demonstrated to result in a heterologous antivenin (fig. 7) containing equine albumin, α_1, α_2, β_1, β_2, IgG, and IgM (Sullivan & Russell 1983). Quantitative protein electrophoresis has demonstrated that the amount of IgG relative to the total protein in the antivenin is only as high as 25% (Sullivan & Russell 1983). Clinically, this antivenin is administered in large intravenous doses of 10 to 15 vials per treatment for a moderate envenomation and the incidence of serum sickness is over 75% in most clinical experiences.

3.2.2 Isolation of Purified IgG(T) Antibody

Investigations over the past 5 years using affinity chromatography techniques have resulted in isolation and purification of the active IgG from equine antisera (Sullivan & Russell 1982). This antibody has been identified as a subclass of horse

IgG and is termed IgG(T) (McGuire et al. 1972; Russell et al. 1985; Sullivan & Russell 1983). *In vivo* and *in vitro* studies with purified IgG(T) have demonstrated its efficacy in neutralising the effects of crotalid venom (Sullivan & Russell 1982, 1983). The use of human *in vitro* leucocyte histamine release assays as well as guinea pig anaphylaxis models has proven the safety of purified IgG(T) in terms of lack of acute release of histamine (Sullivan & Russell 1983). Recently, investigations have resulted in the production of active Fab and Fab_2 fragments from the IgG(T) (Sullivan et al. 1984). Studies with these active fragments are ongoing and may be a promising new immunotherapeutic agent for human envenomations.

The IgG(T) antibody has unique properties. Unlike human IgG, the equine IgG(T) does not fix complement by the classical pathway and has limited precipitability with antigens (McGuire et al. 1979). IgG(T) is also an acidic antibody and contains a high proportion of carbohydrate. In addition, IgG(T) has an extra disulphide bond connecting the high affinity terminus heavy chains (Weir & Porter 1966).

3.2.3 Development of Snake Venom Immunoassays

Another adjunctive immunotherapeutic modality for treatment of snake venom poisoning involves the use of immunoassays to detect venom in cases of human envenomation. The use of an enzyme-linked immunoassay (ELISA) has been refined and its clinical utility demonstrated from the advantage of being able to employ a specific, monovalent antivenin for Australian elapids and other snakes of interest once the venom is detected in biological samples (Hurrell & Chandler 1982; Theakston et al. 1977).

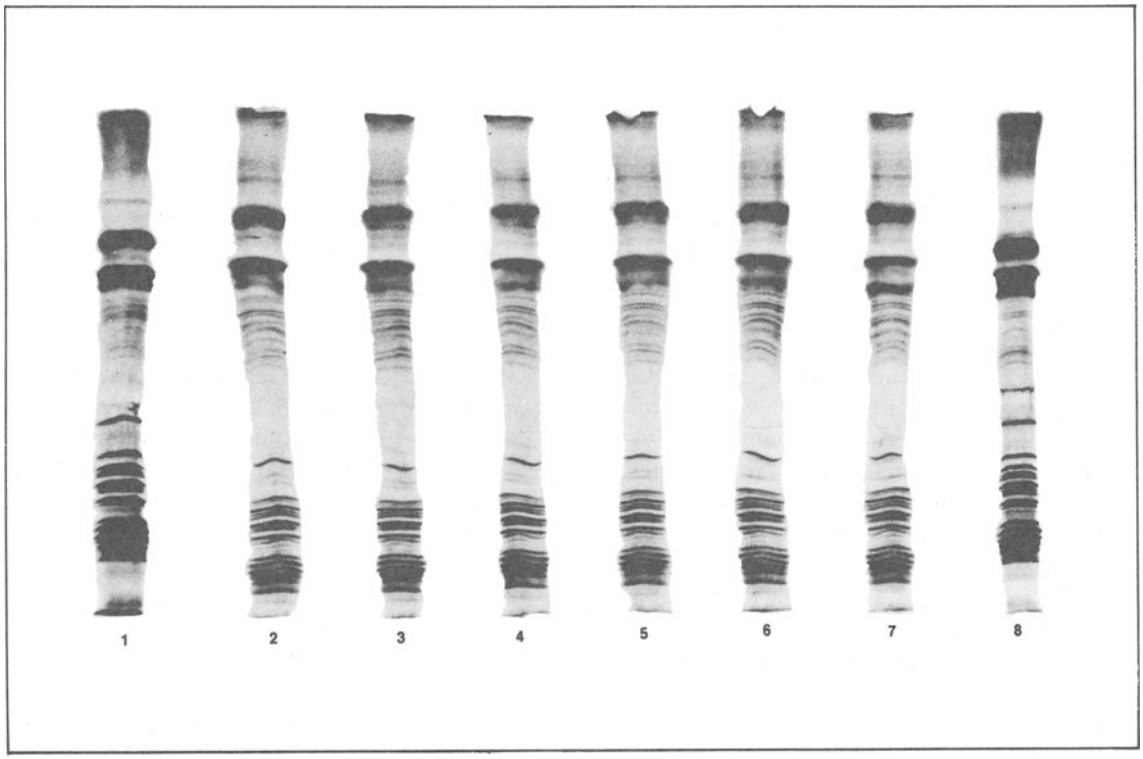

Fig. 6. Multiple protein bands of rattlesnake (*Crotalus molossus*) venom as determined by recycling isoelectric focusing (courtesy of Dr Egen, University of Arizona, Tucson).

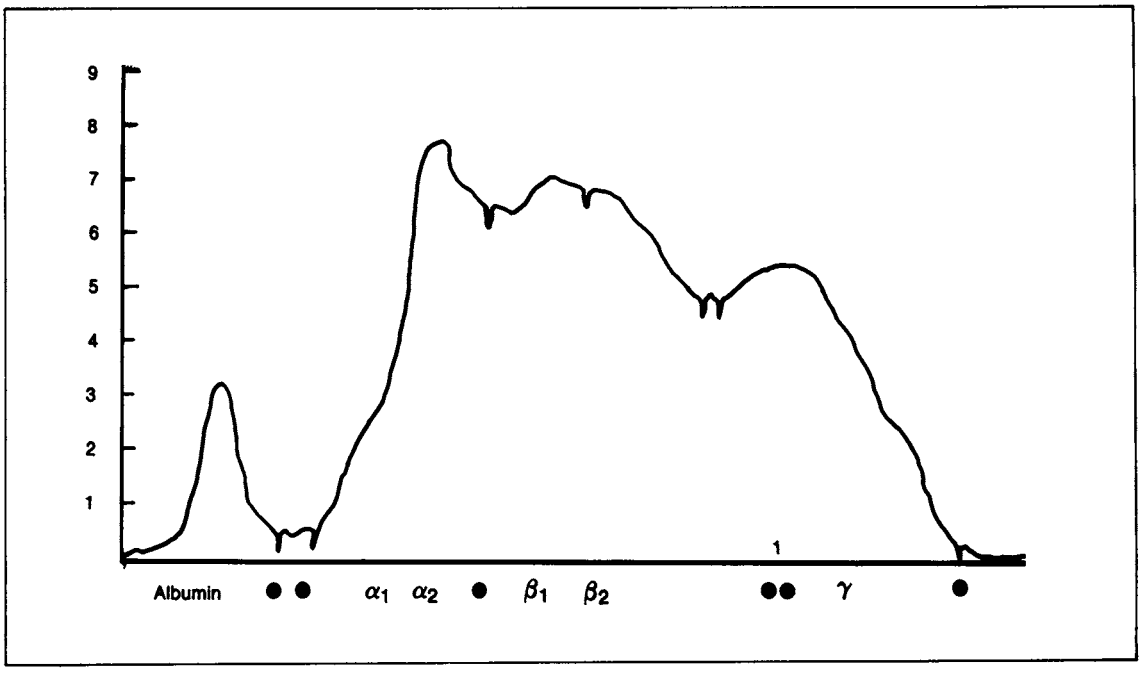

Fig. 7. Quantitative serum protein electrophoresis of Wyeth polyvalent Crotalidae antivenom showing multiple equine proteins in addition to specific IgG(T).

3.2.4 Newer Immunisation Techniques

Further refinements in the clinical use of immunotherapy involves newer immunisation techniques to ameliorate the effects of venom poisoning. Incorporation of venom into lipid envelopes (liposomes) using osmium tetroxide has resulted in a vastly improved method of immunising both animals and humans to produce high titre antibodies to venom proteins (New et al. 1985). The use of these osmicated liposomes allows slow release of venom protein in a non-toxic fashion to be recognised by the immune system. This methodology results in protective antibody titres in animals up to one full year past an immunising dose. Thus in some underdeveloped countries where envenomation is a serious occupational hazard, the use of such an immunisation technique may be beneficial in reducing morbidity and mortality. Also, the utility of this technique relates to the potential to produce a human derived antibody for venom poisoning in the future.

3.2.5 Pharmacokinetic Considerations in Use of Antivenins

Few studies exist on the pharmacokinetics of venoms and antivenins. In one investigation which examined the pharmacokinetics of scorpion venom and antivenin (Ismail et al. 1983), after intravenous administration of radiolabelled partially purified immunoglobulin and whole venom from the scorpion *Androctonus amoreuxi*, the immunoglobulin was found to fit a triexponential equation of a 3-compartment model. There was an initial rapid distribution phase followed by a slower distribution phase with half-lives of 1.1 and 9.6 hours, respectively. The elimination phase of the IgG had a half-life of 43.3 hours. In contrast, the venom was rapidly distributed to tissue sites with a distribution half-life of 5.6 minutes and had an elimination half-life of 6.4 hours.

An important consideration regarding immunotherapy of venom poisoning is that to be effective, antivenin must drastically alter the phar-

macokinetics of the venom. Being a complex poison composed of a multitude of peptides and proteins of greatly varying molecular weights, pharmacokinetic studies of whole venom will only describe average values for distribution and elimination. Also, the smaller molecular weight proteins are renally eliminated *versus* immune cell clearance and catabolism for the larger proteins. Adequate pharmacokinetic studies employing isolated molecular weight ranges of venom have not been performed to date.

4. Approaches to Resolving Antibody Heterogeneity and Immunogenicity

With advanced isolation and purification techniques such as fast-protein liquid chromatography, recycling isoelectric focusing, gel separation, and affinity immunoadsorbent chromatography, polyclonal and monoclonal antibodies can be easily and quickly isolated from foreign protein in antisera. However, the heterologous nature of even monoclonal antibodies remains an impediment to their general use in immunotherapy. Solutions to these problems may lie in recombinant DNA biotechnology and human source antibodies as well as the use of active, high affinity Fab antibody fragments which have a greatly reduced incidence of adverse serum reactions. Human monoclonal antibodies have been produced by fusing human immunoglobulin cells with mouse myeloma cells to generate chimeric human hybridomas (Kaplan & Olsson 1982). However, purely human hybridoma development has remained difficult; but despite this, they will play a major future role in immunotherapy.

Another family of chimeric antibody molecules are being produced in which the variable regions of an animal source are joined to human constant regions. These antibodies, called transfectomas, maintain the antigen binding activity of the hybridoma but are less antigenic due to their human Fc effector region (Morrison 1985). Combinations of variable regions and constant regions from different sources are combined by standard recombinant DNA techniques and can be a source of chimeric antibodies for wide application. Also, antibody in which only the variable region is nonhuman should significantly reduce antigenicity.

5. Future Trends in Immunotherapy for Poisonings

Investigations into the potential clinical value of immunotherapy is ongoing with a variety of drugs and toxins including phencyclidine (PCP) and organophosphate insecticides. Recently, Owens and Mayersohn (1984) demonstrated pronounced alterations in the pharmacokinetics of phencyclidine in tracer studies with dogs. Equimolar doses of anti-phencyclidine Fab fragments were administered to 3 dogs after tracer amounts of radiolabelled phencyclidine were administered and distribution was completed. In these animals, the total serum phencyclidine concentration increased 17, 50, and 56-fold, respectively, along with a drastic decrease in volumes of distribution. Unbound phencyclidine (50%) decreased to $< 1\%$ after Fab administration.

Monoclonal antibodies to the offensive organophosphate soman have been shown to compete with acetylcholinesterase for the toxin resulting in a retardation in the rate of soman inhibition of this enzyme (Lenz et al. 1984). In other studies, reversal of desipramine distribution by anti-desipramine antisera has recently been demonstrated in an animal model (Pentel et al. 1985).

The utility of antibodies in immunodiagnostics and as carriers of imaging agents and cancer therapeutic agents is being further explored along with the development of anti-idiotypic antibodies, which are specific for the combining site of another antibody (Butler 1982; Haber 1982) and can effectively be used as drug receptor probes.

The future of immunopharmacology applied to clinical toxicology is bright. The advent of biotechnological methods for tailor-making antibodies together with advances in human monoclonal biotechnology, and the use of more sophisticated technology applicable to antibody isolation and active antibody fragment production, makes immunotherapy an important part of clinical toxicology's future.

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