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COMMENTARY

DRUG DISPOSITION AND DRUG HYPERSENSITIVITY

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Hypersensitivity is a major complication of drug therapy. It has been estimated that between 10 and 50% of all adverse reactions may have an immunological aetiology [1, 2]. However, accurate estimates of the true incidence of hypersensitivity are not possible because most reports are based on clinical description rather than laboratory analysis. Hypersensitivity reactions are feared by physicians because of their unpredictable nature and potentially fatal outcome. It is therefore essential to identify drugs which, by virtue of either their chemical or pharmacological properties, are likely to provoke hypersensitivity reactions and, also, to identify individuals who may be susceptible to drug hypersensitivity reactions. The purpose of this review is to consider mechanisms of allergic drug reactions and, in particular, to evaluate the role of covalent binding of drugs or their metabolites to macromolecules to generate potentially immunogenic and antigenic forms. A better understanding of the ways in which drugs form such immunoreactive metabolites will improve our chances of predicting and avoiding drug hypersensitivity reactions.

The hapten hypothesis of drug hypersensitivity

Our current understanding of drug hypersensitivity is based largely on the *hapten hypothesis* outlined in Fig. 1. Central to this hypothesis is the assumption that the drug becomes *covalently* bound to a macromolecular carrier such as a protein and that the resulting drug–protein conjugate can be recognised as an immunogen and is then able to stimulate an immune response directed against the drug.

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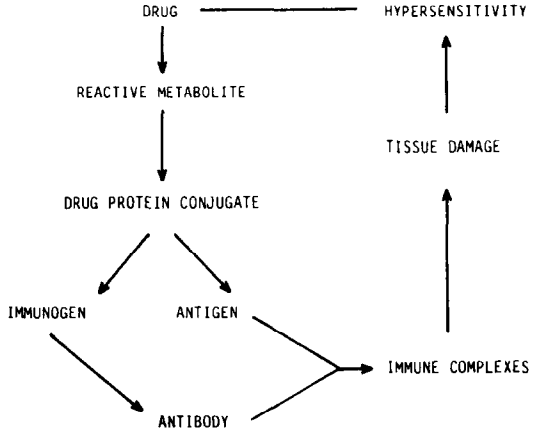

Fig. 1. Scheme illustrating the hapten hypothesis for drug-induced hypersensitivity reactions.

This concept is derived from the classical immunochemical studies of Landsteiner [3], Gell [4] and Eisen [5], and their coworkers, who showed that the ability of low molecular weight chemicals to induce either antibody formation or contact sensitivity is a direct function of their chemical reactivity with nucleophilic groups on proteins, or other macromolecules. The term hapten was coined to describe a substance which is not immunogenic *per se* but becomes immunogenic when conjugated to a macromolecular carrier. The haptenic group may also function as an antigen.

The combination of antigen with either specific antibody or cell type may lead to a hypersensitivity reaction. The nature of the interaction between antigen and the immune system will determine the type of hypersensitivity reaction. Drug hypersensitivity reactions can be conveniently classified according to the general scheme of hypersensitivity proposed by Gell and Coombs [6]:

(1) Specific antigen cross-links specific IgE antibody on the surface of mast cells and basophils to trigger release of chemical mediators such as histamine and leukotrienes.
(2) IgG or IgM antibodies combine with specific antigen associated with the surface of a cell; the cell is destroyed either by phagocytic cells or by activation of the complement system leading to cell lysis.
(3) Interaction of specific antigen with antibodies leads to formation of immune complexes which can induce tissue damage by activation of complement and recruitment of phagocytes.
(4) Lymphokines are released following interaction of antigen with specific T cells; tissue damage is caused by infiltrating mononuclear cells.

The various types of hypersensitivity are not mutually exclusive. A drug hypersensitivity reaction may be predominantly one type, but it may involve a combination of types.

Clearly, drug hypersensitivity may, in theory, present in a wide variety of clinical forms. It is therefore not surprising that many adverse reactions are

Table 1. Drugs against which antibodies have been detected in humans

Drug	Ref.	Drug	Ref.
Alcuronium	[7]	Mianserin	[20]
Amodiaquine	[8]	Morphine	[21]
Captopril	[9]	Nomifensine	[22]
Cephalosporins	[10]	Penicillamine	[23]
Cyclophosphamide	[11]	Penicillin	[24–26]
Dapsone	[12]	Practolol	[27]
Ethynylestradiol	[13]	Quinidine	[28, 29]
Ethylene oxide	[14, 15]	Quinine	[28, 29]
Formaldehyde	[16]	Salicylates	[30, 31]
Halothane	[17, 18]	Suxamethonium	[32]
Methyldopa	[19]	Tubocurarine	[7]

ascribed to an immunological mechanism on the basis of clinical symptoms. However, it is only in rare instances that such a mechanism is *defined* by the detection of either specific drug-induced antibody or specifically sensitised cells of the immune system. A list of drugs for which specific antibodies have been detected is shown in Table 1.

Drugs, such as procainamide and hydralazine, which induce antibodies directed against autologous components but apparently do not induce drug-specific antibodies [33, 34], will not be considered in this discussion.

To understand drug hypersensitivity we must consider under what circumstances a drug (or drug

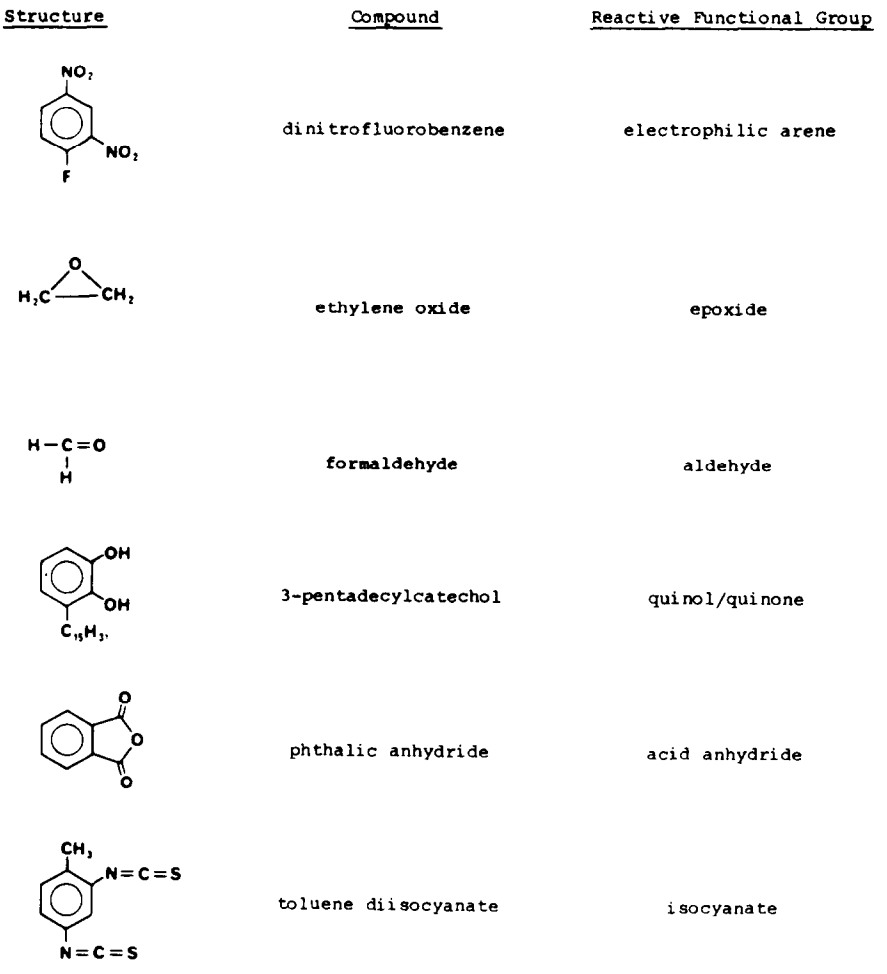

Fig. 2. Examples of compounds that can react directly with proteins and are able to induce an immune response. References: 1 [3]; 2 [14, 15]; 3 [16]; 4 [35]; 5 [36]; and 6 [37].

metabolite) may exert the following immunological functions:

(1) Immunogen: A substance capable of eliciting a specific immune response manifested by the formation of specific antibodies and/or specifically committed lymphocytes. To induce an antibody response an immunogen must possess structurally and functionally distinct determinants for activation of B cells and T cells.
(2) Antigen: a molecule capable of interacting with the antigen-combining site (hyper-variable region) of an immunoglobulin. An antigen may be free in solution or part of a cell membrane. All immunogens are antigens, but not all antigens are necessarily immunogens.
(3) Hapten: a low molecular weight compound that cannot induce an immune response by itself but can act as either an immunogen or an antigen when conjugated to a macromolecular carrier.

Chemical basis of hapten formation

Basic considerations. The first evidence that protein-reactive chemicals produce contact sensitisation by covalent linkage was obtained for chloro- and nitrobenzene derivatives. A marked parallelism between the capacity of such compounds to induce contact sensitivity (a type IV hypersensitivity reaction) in the guinea pig and their chemical reactivity towards aniline was observed [3]. It is now established that a range of chemicals, which react directly with proteins, induce an immune response (T-cell or antibody) in humans and experimental animals, some examples of which are shown in Fig. 2. Nucleophilic groups on proteins include lysyl and cysteinyl residues, and the imidazole and phenol groups present in histidine and tyrosine respectively. The haptenic group formed by small reactive substances generally represents only a portion of the antigenic determinant, which may also encompass part of the macromolecular carrier.

Thus, it can be seen that the essential characteristic required for an agent to function as a hapten is an ability to form stable bonds with nucleophilic groups on proteins, in aqueous conditions. Chemicals that are hydrolysed rapidly will not function as effective haptens. It is also important to note that the chemical structure of the antigenic determinant, comprising the drug (metabolite) and carrier component, may be quite different from that of the parent drug, and this presents a major problem in the detection of anti-drug antibodies. It is therefore important to establish mechanisms by which drugs are able to conjugate to proteins and to determine factors that may influence the process. We will consider separately drugs which react directly with proteins and those that require some form of activation.

Drugs which form protein-conjugates directly

Much of our current understanding of human drug allergy is derived from clinical and laboratory studies of penicillin allergy. It has been shown that antibodies are not directed towards penicillin itself, but towards various haptenic structures which are formed by direct chemical reaction of the drug (or a rearrangement product) and proteins [26, 38]. Penicillins have a reactive structure, with the capacity to bind covalently to proteins and carbohydrates by reactions with nucleophilic amino, hydroxy, mercapto and histidine groups. A number of sites on the penicillin molecule are open to nucleophilic attack and, therefore, a number of antigenic determinants may be formed. Drug–protein conjugation is influenced by pH, certain metals such as copper, and the local concentrations of macromolecules and penicillin. The principal modes of conjugation involve formation of amide bonds, with lysine groups, which can be explained by the inherent chemical reactivity of the β-lactam ring. In addition, opening of the thiazolidine ring exposes a thiol group which can form mixed-disulfide bonds with cysteine or cystine residues on proteins (Fig. 3).

Cephalosporins resemble penicillins in containing a β-lactam ring, but differ in having a dihydrothiazine ring rather than a thiazolidine ring. Cephalosporins are immunogenic when injected in conjunction with Freund's adjuvant. For example, antibodies were successfully raised against cephalothin, when incubated with autologous serum, prior to injection [39]. It is therefore reasonable to suggest that cephalosporins would form a stable cephalosporoyl group, equivalent to the penicilloyl group, upon reaction with protein (Fig. 3) [40]. There is no conclusive evidence, however, for the formation of such an antigenic determinant. The chemistry of hapten formation from cephalosporins has been hindered by the fact that a number of unstable intermediates are formed during aminolysis of cephalosporins [41].

D-Penicillamine is a trifunctional amino acid containing a free sulfhydryl group and a free amino group, and has direct chemical reactivity toward a range of biological macromolecules. The drug can react with biological intermediates that contain a free carbonyl group, via a simple reversible equilibrium process, to form thiazolidine derivatives. It has been established that penicillamine interferes with collagen maturation by chemical reaction with free aldehyde groups in pro-collagen [42]. Penicillamine can also participate in thiol-disulfide interchange reactions between the free sulfhydryl group in cysteine and the cystine groups in proteins [43]. Again, this is a spontaneous equilibrium process that may result in either mixed-disulfide formation or reduction of existing (cystine) disulfide bonds in endogenous proteins. The process is catalysed by transition metals such as copper. Thus, the drug has the capacity to interfere with, and modify, essential self-components in a number of ways; which of these is relevant to the immunotoxicity of the drug remains to be established.

Captopril is an angiotensin-converting enzyme inhibitor used in the treatment of hypertension and congestive heart disease. A number of adverse reactions have been reported, the nature and time–course of which suggest an underlying immunological aetiology [44–46]. Captopril, like penicillamine, contains a free sulfhydryl group, but differs in that it does not contain a free amino group and, therefore, cannot form thiazolidine derivatives with carbonyl compounds. Nevertheless, captopril does form conjugates with plasma proteins via disulfide linkages *in vitro* and *in vivo* [47]. Immunochemical studies have

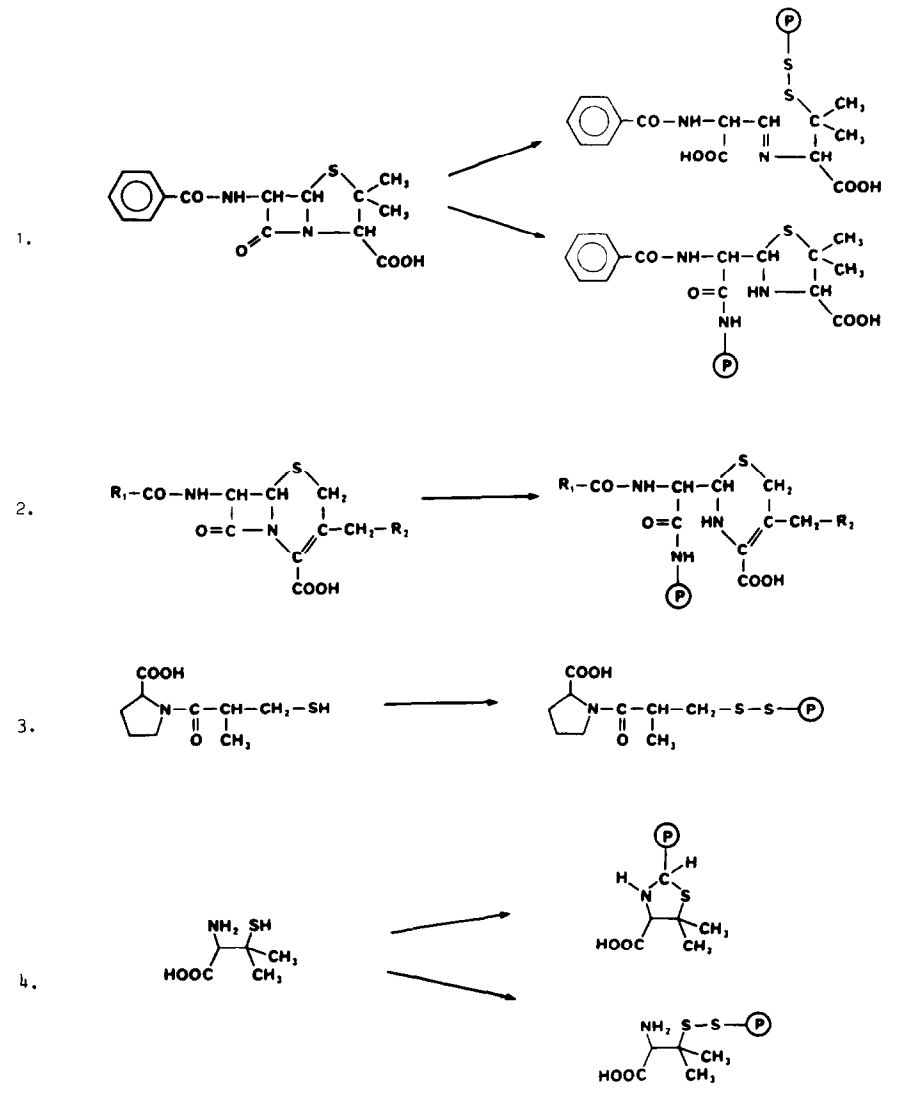

Fig. 3. Chemical reactions by which drugs with direct protein-reactivity can form conjugates. Key: (1) benzylpenicillin, (2) cephalosporins, (3) captopril, and (4) penicillamine.

shown that captopril linked by disulfide bonds to proteins can function as a hapten in humans and experimental animals [9, 48]. Metabolism studies show that conjugate formation is reversible and that the ability of captopril to function as a hapten is dependent on glutathione concentrations and renal function [49].

Formation of drug–protein conjugates from chemically reactive intermediates

Most drugs do not react directly with proteins, and this is to be expected since lipophilic drugs that react indiscriminately with mammalian macromolecular structures are likely to be revealed as either mutagens or carcinogens in preclinical safety evaluation studies. Alternative mechanisms for drug–protein conjugation have therefore been advanced.

For many drugs it is assumed that a chemically reactive metabolite formed *in vivo* will react spontaneously with autologous proteins* and thus produce haptenic groups composed of drug metabolite and a portion of the macromolecular carrier. Although this is an attractive explanation, there is little direct experimental evidence to support it.

During the past 20 years much attention has been focused on the role of chemically reactive metabolites in drug and chemical toxicity [50]. Studies of the relationship between the metabolism and toxicity of compounds such as aromatic hydrocarbons, aromatic amines, 4-ipomeanol and paracetamol have provided firm experimental evidence for the role

* Glossary of terms: autologous protein, a protein obtained from and administered to the same individual animal; heterologous protein, a protein obtained from an animal of one species and administered to an animal of a different species; homologous protein, a protein obtained from an animal of one species and administered to a second animal of the same species; and epitope density, the number of groups of a low molecular weight compound covalently linked to a protein molecule.

Table 2. Postulated chemically reactive metabolites formed from some drugs which produce hypersensitivity reactions in humans

Drug	Postulated type of intermediate	Ref.
Phenacetin	Hydroxylamine	[55]
Sulfanilamide	Hydroxylamine	[55]
Sulfadiazine	?	[54]
Paracetamol	Quinone imine	[56]
Phenytoin	Arene oxide	[57]
Procainamide	Hydroxylamine	[58]
Ethynylestradiol	*o*-Quinone	[59]
Hydralazine	?	[60]
Morphine	Morphinone ?	[61, 62]
Practolol	?	[63]
Halothane	Radicals, acyl halide	[64]

of chemically reactive metabolites as mediators of chemical carcinogenesis and chemical necrosis [51–53]. In contrast, the role of chemically reactive metabolites in immunotoxicity is less well-defined. It is therefore important to consider the role of drug metabolism in hapten formation.

Sulfonamides, which have been widely used as chemotherapeutic agents for the past 40 years, have long been associated with allergy, which is one reason for a decline in their use. Sulfonamides are derivatives of sulfanilic acid and do not possess direct reactivity towards proteins or other endogenous macromolecules. In humans, sulfonamides are metabolised largely by phase II biotransformations such as acetylation and glucuronidation to water-soluble, inert metabolites. Nevertheless, Shear and Spielberg [54] have shown recently that sulfadiazine undergoes metabolic transformation, *in vitro,* to an electrophilic intermediate that is toxic to lymphocytes. However, we have found no evidence for covalent binding of sulfanilamide to either human or rat liver microsomal systems, suggesting that differences in lipophilicity of the side-chain determine the ability of these compounds to be oxidised, and thus form haptens (unpublished data). It has been postulated that unstable *N*-hydroxy-derivatives and nitroso-derivatives may be formed from sulfanilamide in the kidney [55].

A number of drugs, which are thought to produce hypersensitivity reactions and have been shown to form electrophilic metabolites in *in vitro* test systems, are shown in Table 2. In each case, the reactive metabolite is formed in a cytochrome P-450 catalysed reaction. The relative abilities of some of these compounds to conjugate to protein, in a human microsomal test system, are shown in Table 3 (unpublished data).

The ability of a drug to conjugate to proteins *in vitro* does not necessarily reflect the potential of the compound to form haptens *in vivo*, since numerous alternative biotransformations may be available which will either preclude reactive metabolite formation, or deactivate an electrophilic species, once formed. This is well illustrated by our studies on ethynylestradiol, a synthetic estrogen which is thought to function as an immunogen in users of oral contraceptives [13]. Metabolic studies have shown that ethynylestradiol becomes oxidised, via sequential oxidations of the A-ring, to an electrophilic *o*-quinone intermediate, which reacts spontaneously to form stable covalent bonds with nucleophilic groups on proteins [61]. *In vitro*, using human liver microsomes, this process may account for as much as 50% of the drug. However, *in vivo* this process is largely precluded (<2% covalent binding) by extensive and rapid sulfation, glucuronidation and methylation of both ethynylestradiol itself and the pro-reactive metabolite, 2-hydroxyethynylestradiol [65, 66]. In addition, the reactive *o*-quinone intermediate reacts spontaneously with thiols and therefore may be deactivated by conjugation to glutathione. These observations illustrate that hapten formation may be a function of the balance between numerous biotransformations, and that *in vivo* covalent binding studies are essential. Interestingly, chronic administration of ethynylestradiol to the rat leads to an accumulation of circulating drug–protein conjugates [67].

Thus, it can be seen that a number of drugs, which are associated with hypersensitivity reactions of one form or another, have the potential to form haptens as a consequence of enzyme-catalysed biotransformations *in vivo*. This suggests that inter-individual variation in ability and capacity to perform a variety of biotransformations will be an important determinant of drug–protein conjugation *in vivo*. Prediction of hypersensitivity reactions, in relation to the extent of drug–protein conjugation, requires a deeper understanding of factors which influence the immunogenicity and antigenicity of drug–protein conjugates.

Table 3. NADPH-dependent irreversible binding of drugs to human microsomal protein, expressed as percentage of incubated radioactivity

Substrate	% Bound
[^{3}H]Ethynylestradiol	24.3*
[^{3}H]Mianserin	4.8
[^{14}C]Amodiaquine	3.9
[^{14}C]Diphenylhydantoin	0.4†
[^{3}H]Primaquine	0.3*
[^{14}C]Sulfanilamide	Not detectable

Incubations (30 min at 37°) were performed, in triplicate, using liver from the same donor, under the following conditions: 5 mg protein, 10 μM substrate concentration, and 1 mM NADPH.

* 3 mM NADPH.

† 2.5 mg Protein.

Drugs as immunogens

Basic considerations. Much of the information concerning the immunogenicity of various substances has been obtained using animal models designed to be especially sensitive to small doses of putative immunogen. In particular, adjuvants (immune stimulants) and slow release preparations have been used to optimise conditions for an antibody response. Therefore, due caution must be exercised when extrapolating to humans. Important variables that

affect immunogenicity include chemical constitution, degree of antigenic foreigness, and molecular weight. The question of molecular weight is of particular importance when considering the immunogenicity of drugs. In general, molecules of molecular weight of 10,000 or less are poorly immunogenic, while complex proteins of molecular weight of 100,000 or more are usually potent immunogens.

There are, however, exceptions to these simple generalisations. The most widely cited examples are the azo-benzene arsonate derivatives of tyrosine and histidine, which induce delayed cutaneous hypersensitivity in guinea pigs, despite having molecular weights of less than 1000 [68]. Why these particular molecules should be immunogenic is not known. Pentavalent arsenicals may be reduced *in vivo* to trivalent arsenicals which can form covalent bonds with sulfhydryl groups in proteins [69]. It is therefore possible that such compounds undergo biotransformation *in vivo* and eventually function as haptens, but this has not been investigated.

Schlossman and co-workers [70, 71] addressed the question of minimal molecular size for an immune response by investigating a series of oligo-L-lysine derivatives substituted on the alpha-amino terminus by a single 2,4-dinitrophenyl (DNP) group in the guinea pig. It was found that a heptalysyl-DNP conjugate was the smallest molecule that could induce anti-DNP antibody formation, as well as cell-mediated reactions. However, it must be noted that the compounds were administered in an emulsion with Freund's adjuvant, which contains foreign proteins.

The majority of drugs have molecular weights of less than 1000 and are therefore unlikely to function as immunogens *per se*, after normal therapeutic administration. This is perhaps not too surprising, since efficient mechanisms such as metabolism and renal excretion effect the clearance of such compounds without recourse to antibody formation. However, our current understanding of the immune response is not sufficient to completely exclude the possibility of a response to a particular drug or to predict the immunological consequences of chronic drug exposure over a period of several years. Nevertheless, we must at present focus on the immunogenicity of drug–protein conjugates. Factors, that are important variables in this respect include the nature of the carrier molecule, the degree of conjugation (epitope density), and the disposition of the drug before and after conjugation.

Nature of the carrier molecule

Autologous macromolecules in steady contact with immunocompetent cells possess antigenic determinants for which no cells of the host become activated under normal circumstances. Experiments in several species indicate that conjugation of drugs and chemicals to endogenous proteins and cells may initiate an antihapten response. Conjugation leads to the formation of two distinct types of antigenic determinants, the hapten itself and new antigenic determinants that may arise because of structural and conformational modification of the carrier molecule.

The conversion of an autologous tolerated macromolecule or cell into a form which is no longer recognised as "self" is a key step in the generation of an immunogenic hapten–macromolecule conjugate. Dinitrofluorobenzene (DNP-F), a mild arylating agent, has proved to be a valuable tool for investigation of the immunogenicity of haptenated macromolecules. We have also found that this compound is useful as a model for chemically reactive drug metabolites, since it undergoes extensive (95%) detoxification by glutathione, as well as protein-conjugation (5%), after intravenous administration to rats [72]. Søeberg and coworkers [73, 74] have studied the immunogenicity of a range of dinitrophenylated autologous cells and plasma proteins in the pig. They found that pigs could be sensitized for DNP-F-induced delayed (type IV) contact reactions by dinitrophenylated afferent lymph cells and peripheral white blood cells. Dinitrophenylated lymphoid cell membranes and serum albumin were relatively weak sensitizers, while dinitrophenylated globulin was apparently ineffective (Table 4). In our own studies we found that administration of unconjugated DNP-F to rabbits and rats induced specific anti-DNP antibody responses, implicating *in vivo* generation of an immunogenic hapten–macromolecule conjugate [75]. Further investigations in rabbits on the immunogenicity of *ex vivo* generated DNP-autologous plasma protein conjugates revealed that dinitrophenylated autologous albumin is less immunogenic than dinitrophenylated autologous total plasma proteins, suggesting that plasma proteins other than albumin (presumably larger and more complex molecules) constitute the immunogenic carrier [75]. In the case of penicillin, we have found that administration of benzylpenicillin to rats at doses up to 2.7 mmol/kg (1 g/kg) does not lead to an antibody response directed against the

Table 4. Contact sensitivity to DNP-conjugates in the pig*

	Dose (μg/kg)	Response (%)
DNFB	0.03–0.5	100
DNP—serum proteins	6	100
DNP—porcine albumin	20	100
DNP—lymph node cell membranes	20	80
DNP—red cell ghosts	20	100
DNP—thymocyte membranes	20	33
DNP—globulin	20	
DNP—lymph cells	0.005–0.015	40

* Adapted from Søeberg *et al.* [73, 74].

benzylpenicilloyl (BPO) determinant (the major determinant formed from benzylpenicillin), whereas a one million-fold lower dose of BP conjugated to a foreign protein is readily immunogenic [76]. Several other reports have failed to demonstrate immunogenicity of free penicillin in experimental animals [26]. The lack of immunogenicity of penicillin may be explained by the low degree of covalent binding of the drug to plasma proteins observed *in vivo*. In rats, less than 0.006% of the administered dose (27 μmol and 2.7 mmol/kg) is covalently bound to plasma proteins 3 hr after i.v. administration [76]. This is equal to only 4.6 pmol of benzylpenicillin bound per mg of plasma proteins (after 27 μmol/kg) and represents a hapten density of only 2×10^{-4} benzylpenicillin molecules per albumin equivalent. Administration of an equimolar dose of DNP-F leads to an epitope density of 2.8×10^{-2} per albumin equivalent, i.e. covalent binding is approximately 100-fold greater than is the case for benzylpenicillin [75]. The increased covalent binding of DNP-F may account, in part, for its greater immunogenicity compared with benzylpenicillin. However, doses of BP as high as 2.7 mmol/kg (100-fold higher than immunogenic doses of DNP-F) are still non-immunogenic, suggesting that factors other than the degree of covalent attachment may be important for immunogenicity.

Thus, the rather scant information available suggests that the nature of the endogenous carrier molecule and, therefore, the site of conjugation are important determinants of immunogenicity. It has been suggested [26] that haptenation of the membranes of macrophages, which are normally responsible for the presentation of antigen to specific B-lymphocytes may provide a means of short-circuiting the need for immunogen formation. This is an attractive hypothesis that deserves further investigation.

Degree of conjugation—Epitope density

The degree of drug conjugation of a hapten to heterologous or homologous proteins is crucial for the immunogenic potency of the conjugate. Kristoffersen *et al.* [77] found that a conjugate which contained 11 penicilloyl residues per bovine serum albumin molecule induces a significant antibody response in mice after a single injection. However, when the ratio is less than one, as may be anticipated during therapeutic administration of penicillin, there is no detectable response, even after three injections. It must be noted that in this experiment penicillin was conjugated to a foreign protein and administered subcutaneously with Freund's adjuvant, which has a number of ill-defined effects on the immune system. Nevertheless, we obtained similar results after intravenous administration of dinitrophenyl conjugated to autologous albumin without adjuvant to rabbits [75]. A significant antibody response was only observed for an epitope density of 15.

It is pertinent to consider the average epitope densities observed after administration of drugs and model compounds such as dinitrofluorobenzene (Table 5). It can be seen that in all instances the *mean* average epitope density is at least two orders of magnitude less than that required for an immune response. Theoretical calculations have shown that, after intravenous administration of ampicillin (2 g) to man, the maximum mean epitope number on plasma proteins is only 0.0042 and that administration of 20 g should result in, at most 7×10^{-9} g of penicilloylated protein with an epitope number exceeding 5 in the total general circulation [78]. However, the degree of conjugation for individual protein molecules will be dependent on both the rate of reaction of the drug (metabolite) with protein and the rate of drug distribution (dilution). Thus, for a highly chemically reactive drug (or metabolite), drug–protein conjugates with a high epitope density may be formed within the vicinity of the injection (or generation), whereas for a weakly reactive drug, distribution will precede binding to proteins and, therefore, only conjugates with a low epitope number will be formed.

Table 5. Extent of irreversible binding of compounds to plasma proteins following i.v. administration to rats at different doses

Compound	Dose (μmol/kg)	Epitope density
Dinitrofluorobenzene	0.027	1.6×10^{-5}
Dinitrofluorobenzene	27	2.8×10^{-2}
Dinitrofluorobenzene	135	0.1
Captopril	27	1.0×10^{-2}
Penicillin	27	2.1×10^{-4}
Penicillin	2700	3.9×10^{-2}
Penicillamine	27	2×10^{-2}
Ethynylestradiol*	0.017	2×10^{-6}

Epitope density is expressed as the number of moles bound/mole of protein (assuming a mean molecular weight of 68,000). Measurements were made 3 hr after a single injection.

* Measurement was made 24 hr after 22 days chronic administration.

Epitope density may also influence the non-immune clearance of drug–protein conjugates. The hepatic clearance of dinitrophenylated albumin conjugates from plasma is clearly dependent on epitope density. Heavily substituted conjugates are rapidly taken up by Kupffer cells and degraded, and the hapten is excreted as acetyl-lysine-DNP [79].

Nature of the chemical bond

So far, it has been argued that low-molecular-weight compounds must be linked to proteins via a stable, covalent bond in order to act as either an immunogen or a hapten. However, there may be exceptions to this general rule. Nucleic acids and oligonucleotides, which are acidic polymers, can be made into effective immunogens by complexing them with basic protein carriers such as methylated bovine serum albumin [80, 81]. In this instance it would appear that multiple salt linkages are sufficient to carry the hapten through processing by macrophages and to the ultimate recognition stage involving B-cells. The minimum binding force required for immunogenicity is not known, but the reversible forces between drug and serum proteins such as albumin are not sufficient.

It has been suggested that reversible binding between a drug and a specific cellular receptor may lead to localisation of antibodies on cell surfaces, with resulting immune damage. This is the basis of the "innocent bystander hypothesis" suggested by Shulman *et al.* [82] to explain drug-induced immune thrombocytopenia. Some support for this hypothesis has come from the discovery and characterisation of proteins on platelets that have a high affinity for quinine and quinidine. The binding site is thought to be associated with a major membrane glycoprotein, the GP 1b complex, that is not expressed on platelets from patients with the Bernard–Soulier syndrome [83]. It must be noted, however, that the immunogenic forms of quinine and quinidine responsible for the formation of antibodies that bind to the platelets have not been defined. Thus, the possibility of drug–protein complexes, rather than drug–protein conjugates, acting as both immunogens and antigens *in vivo* is possible but has not yet been illustrated.

Drugs as antigens

We use the term antigen to describe the chemical grouping, present on a larger molecule or structure, which combines with the *specific* binding site on an antibody and thus directs the *non-specific* components of the immune system (e.g. complement, mast cells, phagocytes) to the target structure considered foreign or non-self. No assumption is made concerning the identity of the immunogen which stimulated antibody production.

Attachment of a multivalent antigen to antibody is essential for triggering type I and type III hypersensitivity reactions, and it may also enhance type II and type IV reactions. In type I hypersensitivity, cross-linking of IgE antibodies located on the surface of mast cells is required to initiate mediator release. The requirement for multivalent antigens explains why univalent haptens can inhibit allergic reactions to penicillin [84]. Multivalence of the initiating antigen is required in drug-induced serum sickness (type III reaction) which involves deposition of immune complexes.

How can such multivalent conjugates be formed *in vivo*, given the high molar ratio of protein to drug in most circumstances? Direct measurement of epitope density after administration of drug has not yet been achieved and we can therefore only speculate. It is possible that a high local concentration of drug may be achieved by active transport. Thus, penicillin, penicillamine and captopril are concentrated by anion transport mechanisms. Alternatively, localised formation of a chemically reactive metabolite may produce a multivalent drug–protein conjugate.

Conclusions

It is clear from studies on penicillin, and on chemicals such as toluene diisocyanate, that low-molecular-weight chemicals with direct protein reactivity have the capacity to produce hypersensitivity reactions in man. The incidence and type of hypersensitivity are dependent upon the chemical nature of the drug–protein (macromolecule) conjugate, the degree of conjugation, the antigenic characteristics of the conjugate, the disposition of the conjugate, and the immunogenicity of the conjugate. Our current understanding of the relationship between drug disposition and drug hypersensitivity is outlined in Fig. 4. In addition, genetic and host factors will determine individual sensitivity towards a particular, potential immunogen. The information available concerning most of these factors is of a qualitative, rather than a quantitative, nature, and prediction of allergic drug reactions is, therefore, not yet possible.

The role of chemically reactive metabolites in immunotoxicity is still an open question. For some time it has been necessary to assume that drugs which produce allergy, but have no direct protein reactivity, are converted into chemically reactive metabolites which form haptens *in vivo*. While this is an attractive, and one might say convenient explanation, formal quantitative proof for this hypothesis is lacking. There is evidence, that the majority of such drugs

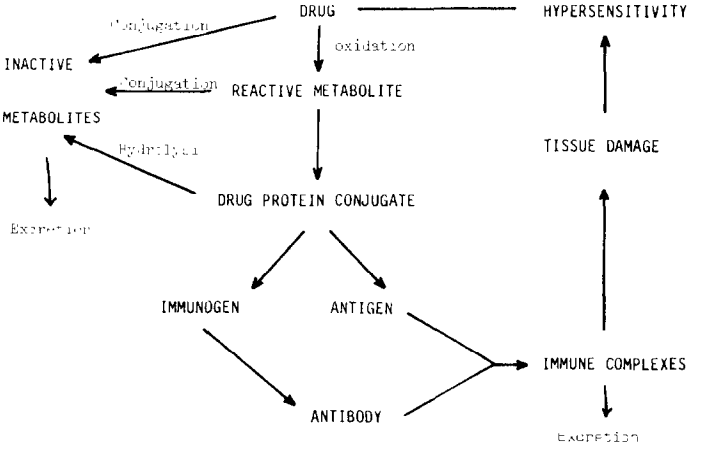

Fig. 4. Scheme showing the relationships between drug hypersensitivity (hapten hypothesis) and the disposition of drugs, drug metabolites and drug–protein conjugates.

can be metabolised to electrophilic species that form covalent bonds with proteins. Therefore, it is possible that the drugs may form haptens *in vivo*. However, evidence in support of this concept is sparse. Theoretically, inter-individual variation in either the rate of formation of chemically reactive metabolites or the rate of detoxication of chemically reactive metabolites could be a major determinant of individual sensitivity to a particular drug. Such variation may also help explain the incidence of drug hypersensitivity and may even help predict the potential hazard of various drugs.

To date, there has been no definitive study on the relationship between inter-individual variation in drug metabolism capacity and the incidence of drug hypersensitivity. There are several reasons why this should be, (1) well defined allergic drug reactions are generally rare and, therefore, prospective metabolism–immunotoxicity studies are a daunting task, (2) retrospective drug metabolism studies are nearly always impossible because of the danger associated with rechallenging with the drug (allergen), and (3) chemically reactive metabolites are difficult to measure because they are (a) chemically unstable and (b) usually minor (<5%) products. Measurement of products derived from chemically reactive metabolites, such as mercapturic acids, may not be relevant, since they are essentially detoxication products. (4) It has proved impossible, in nearly all cases of suspected drug allergy, to provide an adequate animal model.

To overcome these problems we need to develop better experimental models and analytical methods with which to investigate the relationship between drug disposition and immunoreactivity. It will then be possible to undertake prospective studies of the role of metabolism in relation to drug hypersensitivity in man.

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