sensitization detectable circulating antibodies are not commonly found, nor are other common measures of immunological response constantly present.

This rather biased and critical discussion of our knowledge of human hypersensitivity to drugs does suggest that this knowledge is so inadequate that no possibility of designing a screening test which mimics the clinical situation at all closely can as yet be considered. Not surprisingly the experimental work so far published does little to elucidate these obscurities. First examination of the experimental work discloses the surprising fact that the only sensitization phenomenon which has been reliably and indisputably produced in experimental animals is skin sensitization brought about in various ways by direct contact of the drug with the animal's skin, and that the more important blood dyscrasias, liver disorders, asthmas, arteriopathies and arthropathies have not been produced experimentally by drugs known to produce such lesions in human beings. The most illuminating recent work, which will be discussed in more detail by Mr Davies (*below*), is that concerned with contact sensitization to penicillin (De Weck & Eisen 1960, Levine 1960). Here it seems that a metabolite, penicillenic acid, may be important in the production of contact sensitivity and that animals sensitized to this substance show cross-sensitivity to penicillin. Very probably such animals might be used to predict whether a new penicillin would cause sensitization phenomena in individuals already sensitized to older penicillins and a knowledge of this mechanism might be used to predict whether such a new penicillin would, of itself, give rise to sensitization in this way. It would not, however, give any assurance that some other metabolite might not be involved in the case of a new penicillin or that it will not cause any other variety of sensitization phenomena, and of course if one is not interested in penicillin sensitivity the screening test is of no more than considerable academic interest. If we consider the more general implications and accept the thesis that penicillenic acid is the metabolite responsible for human hypersensitivity to penicillin, it becomes of interest to enquire whether other species than man produce this substance. In fact this interesting information does not appear to be available.

Mr Davies will discuss the possibility that in many other cases of hypersensitivity, a metabolite rather than the drug itself may be responsible for the unusual reaction encountered. We know that the metabolism of drugs does vary from species to species and striking examples of this are familiar. Such variation is of importance as a major cause of species differences in both therapeutic and toxic actions of drugs. Indeed in our own laboratories the vast majority of species differences encountered have proved, when adequately investigated, to be due to this cause rather than to differences in the sensitivity of the target organ. If metabolites are indeed important in the genesis of hypersensitivity reactions in man, the work involved in investigating a new drug for its potentialities in this respect is immediately multiplied five- or ten-fold, even if some suitable screening system were to exist; since clearly not only those metabolites known to occur in experimental animals must be investigated but also all conceivable metabolites which might arise in human beings.

It is clear from the foregoing that at the moment there is no prospect whatsoever of setting up even a remotely relevant screening test for the detection of the sensitizing potential in new drugs. Indications exist that in some special circumstances screening tests of limited applicability may be devised but even such tests are probably of doubtful predictive reliability. To this extent, therefore, I have shown that animal tests do have their limitations in this respect and that these limitations are serious. I hope that I have also shown that these limitations essentially arise from our nebulous knowledge of the factors involved in human cases of hypersensitivity and that satisfactory animal tests in this field, as indeed all other fields in which they are used, can only be designed when a sufficient body of knowledge accumulates about the human condition which they are intended to model. Until such knowledge is available it is idle to consider the establishment of such tests.

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Prospects for Animal Tests in Experimental Sensitization to Drugs

by G E Davies BSC (*Macclesfield*)

Dr Paget (p 9) has emphasized the limitations of animal tests in work on drug hypersensitivity and has concluded, justifiably, that at present such tests have no predictive value. This paper sets out to examine critically what has been done and to suggest, with a deliberate bias towards optimism, what more might be done. These considerations themselves have limitations imposed by a lack of definition of the term 'drug hypersensitivity'. I

therefore propose to restrict my remarks to those untoward drug reactions which depend upon antigen-antibody reactions.

Most attempts to induce drug hypersensitivity in animals have employed either application to the skin, or intradermal injection. These techniques are easy to perform and hypersensitivity, should it arise, will become readily apparent at the site of application or injection. These relatively simple methods in the hands of such workers as Landsteiner (1945), Chase, Eisen and Mayer have greatly illuminated our knowledge of the mechanism of sensitization with highly reactive chemicals but they rarely succeed with drugs. There are, however, some exceptions. Mayer *et al.* (1955) were able to sensitize guinea-pigs by repeated intradermal injections of hydrallazine, and Levine (1960) has recently induced contact sensitivity to penicillin G in guinea-pigs. In these experiments Levine used a technique which may have wider application. He dissolved the penicillin in a mixture of ethanol, methyl cellosolve and polysorbate 80 (ECT solvent) and repeatedly applied the solution to the skin of guinea-pigs. We have confirmed this experiment and, using this technique, have shown that there is cross-reactivity between penicillin G and methicillin. The use of this solvent presents a distinct advance: we had previously tried unsuccessfully many times to sensitize guinea-pigs to penicillin G by repeated intradermal injections.

Important as these exceptions are they do not, as yet, establish a general rule. Similar results have not been reported with streptomycin, aspirin or any other important sensitizers.

The reasons for failure of this type of experiment are many and complex. One reason may hinge on the nature of antigenicity. For a drug to function as a complete antigen it must not only be capable of combining with protein but the resulting conjugate must stimulate the production of antibodies. To do this it must reach the sites at which antibodies can be synthesized, or alternatively, it must attract a sufficient number of antibody-producing cells. It may happen, for example, that a compound will conjugate with dermal proteins but, in doing so, become fixed in the skin so that none reaches the nearest draining lymph-node. If the drug-protein complex lacks chemotactic properties it will not have the opportunity of initiating antibody synthesis. If, however, the same complex is introduced into a site already populated with cells already producing antibody to another antigen, the result may be quite different. To test this idea the following experiment was carried out:

Picryl chloride was repeatedly injected intradermally into a group of guinea-pigs until signs of delayed hypersensitivity were apparent. At this point the injections were continued with a mixture of picryl chloride and chlorpromazine. After nine such injections of the mixture, given over a period of three weeks, a rest period of two weeks was allowed and then an injection of chlorpromazine alone was given. All animals showed hypersensitivity not only to chlorpromazine but also to the chemically related promethazine. Animals receiving chlorpromazine alone from the start did not develop hypersensitivity.

Contact sensitivity in guinea-pigs appears to be related to the more general phenomenon of delayed hypersensitivity, as exemplified by tuberculin hypersensitivity, and to depend on antibodies fixed to cells. Some attempts have been made to prepare precipitating antibodies to drugs. Firm chemical combination with protein appears to be necessary for antigenicity of a small molecule. This implies a high degree of chemical reactivity and, although many drugs will form loose complexes with serum albumin, few have the reactivity required for firm combination with protein. Artificial conjugates can be made by chemical manipulation of drugs. Diazotized sulphonamides, for example, have been coupled to serum (Wedum 1942): conjugates have been prepared from aspirin *via* the hydrazide and azide (Harington 1940). Such conjugates have indeed been antigenic but these, and similar studies, have questionable relevance to our main problem.

The fact that most drugs do not form irreversible conjugates with protein has led many authors to postulate that the actual sensitizing substance may be a metabolic product of the drug. Mayer (1954) for example, has shown that guinea-pigs with contact sensitivity to *p*-phenylenediamine will cross-react to its oxidation products *p*-benzoquinone and *p*-quinonediimine. This idea may help to explain the phenomenon of group reactivity when individuals sensitized to a given drug will also react to chemically related compounds. It may also explain hypersensitivity to the first dose of a new drug, the patient actually having been sensitized previously to a drug with a common reactive metabolite.

Further substantiation of this hypothesis, and a much-needed stimulus to work on experimental sensitization in general, has recently been provided by Levine (1960) and De Weck & Eisen (1960). Levine (1960) argued that if two chemicals, A and B, introduce the same antigenic determinant into epidermal proteins then, if one group of guinea-pigs is sensitized by contact with A, and another group is sensitized by contact with B, then the two groups will react indistinguishably

when they are tested at the same time with both A and B. Such compounds he referred to as having allergenic equivalence. He therefore sensitized groups of guinea-pigs to penicillin G and to various degradation products of penicillin. Such equivalence was found with D-benzylpenicillenic acid but not with other compounds. De Weck & Eisen (1960) also selected penicillenic acid as the most likely sensitizer, this time from a consideration of the chemical properties of various breakdown products.

They coupled penicillenic acid to proteins and were able to produce, in rabbits and guinea-pigs, precipitating antibodies specific for the penicillenic acid structure.

One of the most important issues arising from these two papers is the hope they provide that a similar approach with other sensitizing drugs may yield comparable results. A word of caution is, however, necessary.

Both papers showed that penicillenic acid is formed spontaneously in aqueous solutions of penicillin: skin contact with penicillin may therefore, in many cases, result in primary sensitization not to penicillin but to penicillenic acid. There is no indication that penicillenic acid is the only reactive degradation product, it is highly probable that other reactive metabolites may be formed from penicillin *in vivo* and the pattern of metabolic breakdown may well differ in different individuals.

One of the many puzzling aspects of drug hypersensitivity is its relatively low incidence. If sensitization depended simply on the formation of a reactive metabolite which then functioned as a pro-antigen, one would expect a much higher rate. As has been pointed out elsewhere (Davies 1958*a*, *b*) differences in metabolic pathways of drugs in allergic and non-allergic individuals may go some way towards accounting for the allergy. Patients with hypersensitivity to aspirin, for example, may form different metabolites or larger quantities of reactive metabolites than do nonsensitive people.

The possibility of the sensitizing agent being a reactive metabolite has been dealt with at some length since it provides one of the few glimmers of light in a poorly illuminated field, but it must be admitted that the hypothesis is completely without proof.

So far I have dealt only with allergic reactions made manifest by the appearance of precipitating antibodies or dermal reactivity. There are no reports of the experimental production of other manifestations of drug hypersensitivity as seen in man.

It must be admitted that at the moment the prospects for animal experiments are not very encouraging. Further major advances may well await the appearance of some entirely new concept, but, lacking this, consideration must be given to the lines research might take to provide background for such a concept.

First of all, a fundamental criticism must be dealt with. It is often claimed that, because the incidence of sensitivity to a given drug in man is low, extremely large groups of animals must be used in the hope that some few of them may become sensitized. Is this necessarily so? Only a small proportion of the human population develops allergy to foreign proteins but nearly every guinea-pig can be anaphylactically sensitized. It therefore seems most likely either that failure of sensitization, in both man and animals, is failure of the drug to form an effective antigen, or that the drug forms a weak antigen but the methods used for demonstrating its antigenicity are inadequate.

Consideration of the potential sensitizing ability of a new drug must take into account the following: Does its chemical structure suggest that firm chemical combination with protein is possible? Are reactive metabolites known, or can they be postulated? Does its structure resemble that of a drug already known to be a potent sensitizer?

If one or more of these questions can be answered affirmatively then the following course of action is suggested: (1) Inject the drug or its metabolite intradermally into a group of at least 20 guinea-pigs repeatedly over a period of at least three weeks. (2) Dissolve the drug or metabolite in a solvent such as ECT (Levine 1960) and apply to the skin repeatedly for three weeks.

If three such courses, with intervening rest periods of two weeks, induce hypersensitivity then it may be predicted, with some degree of confidence, that the drug will produce contact sensitivity in man. Further evidence might be gained by attempting to induce the formation of circulating antibodies in rabbits and guinea-pigs, using as antigen a conjugate of the drug or metabolite with protein. Feeble antigenicity may be enhanced by the use of adjuvants. For measurement of antibodies in low titre some sensitive method such as hæmagglutination or passive cutaneous anaphylaxis may be used. It may also be helpful to examine the treated animals for other evidence

of possible hypersensitivity such as reduction in serum complement following a dose of antigen or antigen-induced changes in the blood picture.

The use of animals already sensitized to some other simple chemical may be advantageous, as I have described for chlorpromazine. Finally, consideration might be given to the possibility of using animals selectively bred for the easy development of sensitivity. Chase (1941) has bred guinea-pigs which are very readily sensitized to picryl chloride. It would be interesting to breed animals which can be readily sensitized both to penicillin and to picryl chloride.

Positive results in any of these tests would indicate special caution if the drugs are to be given to man.

Unfortunately, however, negative results would not remove the need for this caution. Nevertheless it may be anticipated that information collected in this way would pave the way for an eventual correlation between sensitization in animals and in man.

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Mechanism of Development of Drug Sensitization of the Skin

by Professor H O Schild MD DSC PHD (*London*)

It has been known for a long time that the human skin can become sensitized to simple chemical substances and the patch test of Jadassohn is used to test for this sensitization. Experimental contact sensitization in guinea-pigs was first produced with neosalvarsan (Frei 1928) and soon afterwards with phenylhydrazine, paraphenylenediamine and primula extract. In an early paper Bloch & Steiner-Wourlisch (1930) showed that application of primula extract to a patch of guinea-pig skin was followed about five days later by generalized hypersensitivity to the extract of the whole skin. These workers also found that repeated administration of the extract produced no desensitization and that the sensitization could not be transmitted by the serum or wheal fluid from a sensitized animal.

Landsteiner & Jacobs (1935) used dinitrochlorobenzene (DNCB) and picryl chloride (PC) to produce sensitization in guinea-pigs, and most subsequent work on experimental contact sensitization has been carried out with this type of compound. These substances when applied to the surface of the skin produce hypersensitivity of the entire skin after a few days, in the same way as primula extract. The reaction produced after the second application begins to arise after a few hours and is maximal after about twenty-four hours, resembling in its general appearance a typical delayed reaction such as the tuberculin reaction.

PC and DNCB are toxic compounds and if administered in a sufficiently high concentration produce a primary toxic irritation of the skin which macroscopically is indistinguishable from the allergic reaction produced by smaller doses in a sensitized guinea-pig. Nevertheless microscopic examination reveals differences between the two types of reaction. In particular the allergic reaction is characterized by a strong infiltration with mononuclear cells which is absent in the primary toxic reaction.

One of the characteristic features of contact sensitization of the skin is that it can be produced most effectively by applying the antigen to the skin itself. Intramuscular, intraperitoneal, or even subcutaneous application of the antigen is usually less effective. A second feature of the contact reaction is that it is produced most effectively by application of a simple low molecular 'hapten'. Haptens which are conjugated to proteins before being injected are relatively ineffective. On the other hand simple haptens as well as conjugated haptens produce an anaphylactic sensitization when they are injected intraperitoneally. Although the same substance is effective in producing 'delayed' contact sensitization and anaphylactic sensitization, and although the two conditions often occur together, they are clearly separable. Thus it is possible to desensitize an animal against anaphylactic reactivity without diminishing its delayed skin hypersensitivity. Animals which have been passively sensitized by injection of circulating antibody reactive to picryl protein show typical Arthus reactions when treated with PC intradermally, but no delayed skin reactions (Benacerraf & Gell 1959).