REVIEW ARTICLE

THE MECHANISM OF CONTACT SENSITISATION

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It has long been known that human skin can be sensitised by contact with simple chemical substances and the patch test of Jadassohn was used as a clinical method to detect this kind of sensitisation. Experimental contact sensitisation in guinea-pigs was produced for the first time in 1928 by means of neosalvarsan (Frei, 1928) soon to be followed by similar experiments with *para*-phenylenediamine (Mayer, 1931), phenylhydrazine (Jadassohn, 1930) and primula extract (Bloch and Steiner-Wourlisch, 1930). In a remarkable investigation Bloch and other (1930) demonstrated that the local application of primula extract to guinea-pig skin was followed a few days later by sensitisation of the entire skin. They showed that repeated application of the extract did not produce desensitisation and that the sensitisation could not be transmitted by means of serum or wheal fluid. Earlier (Bloch and Steiner-Wourlisch, 1926) these same workers had shown that a sufficiently large dose of primula extract would sensitise almost 100 per cent of a group of human subjects, thus disposing of the idea that hypersensitivity could be achieved only in a small proportion of "idiosyncratic individuals".

Landsteiner and Jacobs (1935) used substituted benzene derivatives, for example, dinitrochlorobenzene (DNCB) and picryl chloride (PC) to induce contact sensitisation in guinea-pigs, and most of the subsequent work in this field has been carried out with this type of compound. Following the intracutaneous or epicutaneous application of DNCB or PC to guinea-pig skin, a generalised skin hypersensitivity develops on the 5th to the 9th day. If at this stage a second application is made elsewhere on the surface of the skin, a pinkish reaction on a slightly swollen background begins to arise at the second site after a few hours; this reaction becomes maximal after 24–48 hr. The individual susceptibility of guinea-pigs towards contact sensitisation varies. Some guinea-pigs cannot be sensitised at all, and strains of markedly different genetic susceptibility have been isolated (Chase, 1941).

The main site of the contact sensitisation reaction is in the basal layers of the epidermis. Although there is a superficial resemblance between the primary toxic effect of a large dose of PC and DNCB in a non-sensitised guinea-pig and the effect of a much smaller dose of the same compound in a sensitised guinea-pig the histological character of the lesion in the two instances is different (Jadassohn, Bujard and Brun, 1955; de Weck and Brun, 1956; Fisher and Cooke, 1958a). The primary toxic response is characterised by degeneration of epidermal cells with moderate leucocytosis, whilst the allergic response is characterised by a rapid massive extravasation of mononuclear cells migrating in trails directly into the

epidermis. The cell extravasation may be followed by vacuolisation and vesiculation leading to a disruption of the epidermis and to cellular death and exfoliation.

Delayed Hypersensitivity and Anaphylactic Hypersensitivity

Besides producing delayed sensitisation simple chemical substances can also produce a typical anaphylactic sensitisation characterised by Dale-Schultz reactions, "immediate" wheal and flare and circulating antibody. It depends largely on the route and manner of administration of the antigen which type of sensitisation prevails.

Anaphylactic sensitisation tends to occur after the intraperitoneal injection of a simple chemical substance (hapten, proantigen) or of a conjugate produced by the reaction of a hapten with protein *in vitro*. Delayed skin reactivity occurs after the application of haptens to the skin—either to its surface or intradermally—but not usually after intraperitoneal injections (Chase, 1954). However, simple haptens can produce delayed skin reactivity when they are injected intraperitoneally together with killed tubercle bacilli (Landsteiner and Chase, 1941) or their purified wax fraction (Raffel and Forney, 1948). The mode of action of tubercle bacilli in favouring skin sensitisation by intraperitoneal injection of low molecular chemical substances is not clear. Mayer (1956) has suggested that the effect of mycobacteria in promoting delayed reactivity may be due to accumulation in tubercles of collagen (Rich, 1951) with which the hapten combines. In support of this view he showed (Mayer, 1957) that a pro-collagen injected with PC produced a similar adjuvant effect to tubercle bacilli in promoting delayed skin reactivity.

Most workers have found that conjugates made by allowing simple haptens to react with protein *in vitro* do not produce delayed reactivity in guinea-pig skin even when administered by intracutaneous injection (Gell, 1944; Chase, 1954; Eisen, Kern, Newton and Helmreich, 1959). Injection of picryl conjugates may, however, be followed by the appearance of delayed hypersensitivity to the protein carrier in the absence of hypersensitivity against the haptenic group (Benacerraf and Gell, 1959a). Only exceptionally have delayed sensitisations by protein conjugates been reported through the administration of large doses of picryl proteins (Benacerraf and Gell, 1959b) or of conjugates made by combining PC with homologous erythrocyte stromata (Landsteiner and Chase, 1941). It is, however, difficult in these experiments to exclude entirely the possibility that traces of unconjugated PC may have been responsible for the sensitisation.

Although conjugates generally fail to produce delayed sensitisation of the skin they are often highly effective in producing serum antibodies capable of inducing anaphylactic sensitivity, as shown by a positive Dale-Schultz reaction, generalised anaphylaxis in the guinea-pig or Praussnitz-Kustner reactions after the serum is transmitted to normal guinea-pigs. Highly reactive compounds such as acyl chlorides, which presumably combine with proteins as soon as they are injected, are also highly effective

in producing anaphylactic sensitisation. When an animal has been sensitised by means of acyl chloride intraperitoneally, the injection of an acyl protein into the skin produces an immediate flare and wheal reaction (Landsteiner and Jacobs, 1936). Gell, Harington and Michel (1948) have tested the antigenicity of certain highly reactive compounds and compared this with their hydrolysis rate and their reactivity with amino groups. They concluded that factors favouring antigenicity of the immediate type are, a relatively slow rate of reaction and a high conjugation to hydrolysis ratio. Thus compounds which are hydrolysed with extreme ease would be expected to be relatively ineffective in forming antigens *in vivo* whereas more stable compounds will not only be more likely to react with amino groups before hydrolysis occurs but they may also in part survive unchanged till they are taken up by cells in which they may find an environment more suitable for conjugation.

The same low molecular weight substances may induce anaphylactic sensitisation and delayed skin contact sensitisation. Nevertheless the two types of sensitisation are independent and separable when they occur together. Thus animals showing both anaphylactic and contact sensitisation can be completely desensitised to the anaphylactic type of sensitisation either spontaneously or experimentally without in any way losing their delayed cutaneous reactivity (Landsteiner and Chase, 1937; Raffel and Forney, 1948). Animals which have been passively sensitised by injection of circulating antibody reactive to picryl protein show typical Arthus reactions when treated with PC intradermally, but no delayed reactions (Benacerraf and Gell, 1959b). Furthermore the presence of precipitating antibodies confers no protection against delayed reactivity (Gell, 1944).

Sensitising Activity and Chemical Reactivity

Landsteiner and Jacobs (1935) investigated a number of chloro- and nitro-derivatives of benzene for their skin sensitising effects and concluded that a close connection existed between skin sensitising capacity and the possession of labile Cl or NO_2 groups. They considered that active compounds carried out substitution reactions and attached themselves to the basic groups of proteins. Brownlie and Cumming (1946) later confirmed that aromatic skin-sensitising nitro-compounds formed condensation products with amino-acids *in vitro*. Some active skin sensitisers are themselves unreactive with proteins but they may be metabolised to reactive derivatives. Examples are picric acid which possesses nitro groups which are not readily detached, and also *para*-phenylenediamine and the polyhydric phenols contained in poison ivy. The latter are probably oxidised in the body to quinones which then react with proteins. Eisen, Orris and Belman (1952), have pointed out that mere adsorption on proteins is insufficient for contact sensitisation and that formation of a covalent link is probably necessary.

Protein binding appears to be necessary both for the induction of sensitisation and for eliciting a reaction. Eisen and others (1952) investigated eight dinitrophenyl derivatives for their capacity to elicit delayed

skin reactions in guinea-pigs sensitised by dinitrofluorobenzene. The four derivatives which produced skin reactions were all capable of combining with proteins whilst the remaining four compounds which produced no skin reactions also failed to combine with proteins. Protein binding was demonstrated in two ways: firstly, by allowing γ-globulin to react with the haptens *in vitro* and measuring protein-binding spectroscopically; secondly, by treating guinea-pig skin with the haptens *in vivo* and identifying the formed dinitrophenyl amino-acids chromatographically after excision of the skin and acid hydrolysis. It was found that each of the active compounds had combined with the ϵ-NH_2 group of lysine to form dinitrophenyllysine. Another group of active skin sensitising compounds were shown to react with the —SH and —S—S— groups of cysteine and cystine in hair and epidermis (Eisen and Belman, 1953). Conjugation of hapten with proteins in the basal layer of the epidermis is considered by Eisen and Tabachnik (1958) to be an essential step in the process of eliciting a contact sensitisation reaction.

If the formation of protein conjugates *in vivo* underlies both "immediate" and "delayed" sensitisation some kind of explanation is required to account for the relative ineffectiveness of pre-formed protein conjugates in causing contact sensitisation; and also, the failure of intraperitoneal injections of simple chemical substances (haptens) to induce contact sensitisation unless they are combined with an adjuvant such as tubercle bacilli.

An interesting explanation of these anomalies has been put forward by Mayer (1956) who suggested that the sensitising properties of haptens were closely related to their tanning properties, that is their ability to form cross links with adjacent protein macromolecules. Haptens might form cross links with different types of protein according to their site of injection: with fibrous proteins of the keratin and collagen groups when in contact with the epidermis, and with globular proteins of the albumin and globulin groups when injected intraperitoneally. In this way different complete antigens may be produced: in the epidermis, rigid, oriented, difficultly soluble or insoluble antigens which could act as templates for equally insoluble sessile antibodies; in the peritoneum, soluble antigens possessing globular carrier proteins on which the humoral, soluble antibodies are moulded. Mayer attributes the effect of mycobacteria in promoting delayed reactivity to the high collagen content of tubercles as already discussed.

An entirely different explanation of the low effectiveness of protein conjugates in contact sensitisation is suggested by some work of Eisen and others (1959). These authors found protein conjugates consistently ineffective in producing delayed sensitisation even when the protein was derived from hair or epidermis. They then incubated haptens and their corresponding protein conjugates with lymph nodes *in vitro* and measured uptake. The simple haptens were concentrated 30 to 300 times inside the lymph node cells whilst the protein conjugates were not concentrated at all. This suggests that contact sensitisation may depend on an initial uptake of hapten by lymph node cells followed by intracellular conjugation of the hapten with protein.

Development of Contact Sensitisation

When DNCB is applied to the skin of a guinea-pig some of it combines with skin protein. If the skin is extirpated 24 hr. later about half the material still present is in a combined form and of this 99 per cent is present in the epidermis mostly combined with the NH_2 groups of lysine residues (Eisen and Tabachnik, 1958). Some of the DNCB is absorbed into the circulation and excreted in the urine, but the strategic site for the induction of contact sensitisation is the local lymphatic system.

Seeberg (1951) has shown that the skin can be sensitised to DNCB by injecting the compound directly into an exposed lymph gland under complete avoidance of the skin. Frey and Wenk (1957) carried out a series of interesting experiments with skin stumps connected with the body by blood vessels and nerves. The lymphatic system of the skin stumps was either left intact or removed. DNCB produced initial sensitisation of the rest of the skin only when applied to a stump in which the lymphatic system was left intact. On the other hand the local lymphatic system was not required for the further maintenance of the sensitisation. If the regional lymph nodes were extirpated within 48 hr. of primary contact no sensitisation at all occurred but if the extirpation was carried out later there was an increasing incidence of sensitisation. If the extirpation took place 9 days after the primary contact all the experimental animals became and remained sensitised, suggesting that at this stage antibody production occurred also in lymph glands removed from the site of application.

The subsequent generalising of sensitisation most probably takes place through the blood stream as indicated by the following findings: firstly, skin sensitisation to DNCB can be transmitted by parabiosis (Haxthausen, 1943b); secondly, in cross-transplantation experiments with uniovular human twins of which one was sensitised to DNCB and the other unsensitised, a skin transplant from the unsensitised to the sensitised twin became itself sensitised whilst a transplant from the sensitised to the unsensitised twin lost its sensitisation (Haxthausen, 1943a); thirdly, in Frey and Wenk's (1957) experiments the application of DNCB to a remote part of the skin produced sensitisation of an isolated skin flap even when the stump had its lymphatic system removed.

Cellular Transfer of Contact Sensitisation

Contact sensitisation cannot be transferred by even very large quantities of plasma (Haxthausen, 1951), but it can be transferred by the cellular elements of blood as was first shown by Landsteiner and Chase (1942). These workers sensitised guinea-pigs by the intraperitoneal injection of PC bound to stromata of guinea-pig erythrocytes mixed with a suspension of dead tubercle bacilli (this treatment resulted in a strong hypersensitiveness of the skin to PC). Repeated intraperitoneal injections of killed tubercle bacilli produced a peritoneal exudate containing leucocytes and lymphocytes. Exudate cells were collected from several donors and after centrifuging and washing they were injected into normal guinea-pigs.

Two days later the application of PC to the skin of the recipients induced a typical delayed erythematous reaction. A few days later the hypersensitivity subsided. The clear supernatant from the exudate failed to transmit the hypersensitivity.

Successful transfer of contact sensitisation has also been achieved with cells from spleen and lymph nodes (Chase, 1946), thymus (Haxthausen 1947), thoracic lymph duct (Skog, 1956), and blood (Haxthausen, 1951). The number of cells required for a successful transfer is about 5×10^8. Active peritoneal exudates produced by the intraperitoneal injection of paraffin contain mainly mononuclear cells. Exudates produced by the injection of saline, containing predominantly polymorph-nuclear leucocytes, are inactive (Haxthausen, 1951). Earlier work seemed to indicate that the transfer factor was a cell-fixed antibody which could not be extracted and was present only in freshly prepared cells. Thus cells damaged by freezing and heating (Chase, 1941) prolonged standing (Nilzen, 1952) and haemolysis (Skog, 1956) were found inactive. More recently, however, Jeter, Tremaine and Seebohm (1954) have reported that peritoneal exudate cells disrupted by sonic oscillations are capable of transferring contact sensitisation to DNCB. These observations have been confirmed (Turk, 1961). Jeter, Laurence and Seebohm (1957) reported that the active extracts contained a component resembling an α-1-globulin which was absent in similarly prepared extracts from normal cells.

Mechanism of Delayed Skin Reaction

The role of cells of the mononuclear series in contact sensitisation seems clearly established by transfer experiments and it is also shown by the massive extravasation of lymphocytes after the application of allergen to sensitised skin. The delay in the response can be explained, in part at least, by the time required for accumulation of cells at the site of administration of the antigen. However, this is probably not the whole explanation of the delay if the tuberculin reaction can be taken as a guide. Thus Metaxas and Metaxas (1955) found that when tuberculin sensitised cells were injected intradermally together with tuberculin the characteristic delay of the tuberculin reaction was still present. The delay is thus probably in the reaction itself.

Very little is known of the pharmacological and biochemical events which underlie the delayed skin reaction. It has been suggested that the sensitised cells act simply as carriers of antibody which is subsequently transferred to tissue cells. In that case the antigen would presumably be reacting with tissue cells which in turn would be releasing pharmacologically active substances responsible for the delayed reaction. Another suggestion is that sensitised mononuclear cells metamorphose into sensitised epithelial cells (Andrew and Andrew, 1949). Perhaps the most probable assumption is that the antigen reacts with sensitised mononuclear cells which are attracted to the skin but the mechanism of this reaction is unknown. Indeed any reaction scheme between cell-bound antibody and protein-bound hapten presents formidable theoretical difficulties which so far have not been resolved.

Another probable assumption is that as a further step in the reaction sequence pharmacologically active substances are released which cause the delayed vasodilator response. Contrary to earlier views that histamine is implicated only in "immediate" anaphylactic reactions (Mongar and Schild, 1962) evidence has recently been forthcoming which suggests that histamine may also play a part in delayed hypersensitivity. This evidence is rather indirect and derives from two sources. The first: in a typical "delayed" reaction such as the tuberculin reaction the histidine decarboxylase activity of the skin is increased (Schayer and Ganley, 1961). Schayer (1959) has suggested that a protracted release of newly formed histamine may be responsible for the vasodilatation. The second: the histamine content of guinea-pig skin rises during "delayed" skin reactions.

The increase begins about 3 hr. after administration of the antigen and is maximal after 24 to 72 hr. The histamine increase is correlated with the infiltration of mononuclear cells but it cannot be explained simply by the importation of histamine by these cells since the increase of histamine considerably exceeds the amount present in the infiltrating cells. The increased histamine content may thus be due partly to increased histamine formation (Inderbitzin, 1961). Fisher and Cooke (1958b) found that the histamine content of the skin increased in a primary toxic reaction due to DNCB as well as in an allergic reaction due to this same substance but it was much greater in the allergic reaction. These authors are of opinion that histamine functions as an accelerator of repair processes in the skin rather than as a cause of the dermatitis. Other pharmacologically active substances, for example polypeptides, may also be involved in the vascular reaction of delayed hypersensitivity, but so far their presence has not been demonstrated, possibly due to a lack of suitable experimental procedures for detecting them. Such substances could be present in a preformed state in the infiltrating cells or they could be formed as a consequence of the reaction of sensitised cells with antigen.

Lymph node cells from guinea-pigs sensitised with DNCB exhibit changes in their metabolic pattern, for example, they incorporate methionine and orthophosphate at an increased rate. These metabolic changes are not directly correlated with cell proliferation and it has been suggested that they may be related to the formation of an intracellular phosphoprotein antibody (Kern and Eisen, 1959).

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