Allergy, hypersensitivity and cosmetics

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Synopsis—The difficulties of immunological nomenclature are discussed, the term ALLERGY defined and the various types of HYPERSENSITIVITY reactions are listed and characterized. Evidence for the association of Type I and Type II hypersensitivity reactions with COSMETICS is discussed. A table of cosmetic ingredients which have been implicated as SENSITIZERS are given. PREDICTIVE PATCH TESTS for contact sensitizers on GUINEA-PIGS and man are evaluated. The difficulties of testing for ALLERGENS likely to produce Type I hypersensitivity are discussed. *IN VITRO* tests for sensitizers are mentioned. The failure of all standard tests in the detection of weak sensitizers is emphasized.

INTRODUCTION

Beautifying aids have always been used extensively by women and men from the earliest times and most women would regard them as essentials rather than luxuries. That some hazard might attend their use is accepted but whilst a dermatitis contracted from the use of an ingredient obtained by the woman herself may be regarded by her as a natural hazard, a similar effect resulting from a manufactured product containing the offending ingredient will be viewed in an entirely different light.

The manufacturer will be held directly responsible for any inconvenience caused or for any deterioration in her appearance. At the least he faces the prospect of the loss of her custom and possibly that of her friends as all his

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products will tend to become suspect; at the worst he may face legal action, compensation payment and adverse publicity. For his own sake, as well as that of the public, the wise merchant tests his wares as carefully as possible.

Considering their wide use and abuse, cosmetics cannot be accused of being a major source of potent allergens but they can be suspected of being a possible major source of weak sensitizers. The problem of detecting these satisfactorily and avoiding or minimizing their use is a major one for the cosmetic industry.

Nomenclature

Every craft and discipline has its own peculiar language or jargon which arises from the necessity for meaningful, but succinct communication between initiates. New words are coined to define new concepts, principles or discoveries and existing words are given specialized meanings, often at variance with everyday usage. The new language does not always help to achieve clarity of thought or expression among its exponents and almost always causes confusion among laymen. The language of immunology is no exception and the difficulty experienced by many immunologists in presenting their ideas both to colleagues and laymen and the concomitant difficulty experienced by many non-immunologists in understanding the subject is largely semantic.

That confusion obviously exists as to the exact meaning of such words as allergen, sensitizer, hypersensitivity and allergy is evidenced by such statements as 'Casein is not a primary irritant and . . . has not been found to possess sensitising properties. It appears to be innocuous, though a few persons are allergic to it', (1) which appear even in reputable text books. Allergy (whence allergic) is an example of a new word describing a new and well-defined concept of altered reaction by an animal body to a foreign substance. Although it is now frequently used in a way not intended by its inventor von Pirquet (2), it always has a clear and specific meaning, unconfused by any prior concept. Thus most readers would readily grasp that some persons react abnormally (i.e. are allergic) to casein. Sensitivity, sensitive implied by 'sensitizing properties' however, have a common usage quite unrelated to their specific usage by immunologists.

The statement that someone is sensitive or hypersensitive to, say, oranges can mean that person simply feels differently from the majority of of his fellows to the smell, taste or even colour of oranges—in other words he does not like the fruit and avoids it. To an immunologist, however, the

phrase 'sensitive to oranges' would imply a definite, physical change in the person's tissues in response to contact with the fruit associated with definite symptoms. In this instance the orange would be a sensitizer. In this context sensitizer and allergen are synonymous. The individual is allergic/hypersensitive to a sensitizer/allergen in the orange. The quotation read, in this light contradicts itself since a substance said not to possess sensitizing properties—which implies it is not a sensitizer—is also said to produce allergic reactions which means that it must be a sensitizer.

The phrase often heard that a person's skin is 'very sensitive' often only implies, for example the increased liability of a red head to develop sunburn and is quite unrelated to any abnormal immunological reaction—although the phrase may be also used in such a context. However, this common usage of 'sensitive' has been adopted by some dermatologists in describing skin reactions to irritants as well as allergens.

It is especially regrettable that the term 'photosensitivity' in dermatological parlance embraces phototoxic *and* photoallergic reactions and substances eliciting these are referred to collectively as photosensitizers. For this reason, only, the terms allergen or *contact* sensitizer are to be preferred for the sake of clarity to the unqualified term 'sensitizer'.

Hypersensitivity

Immunological reactions are mediated by the lympho-reticular system and are primarily designed to protect the individual from potentially noxious foreign antigens contained in bacteria, viruses, food and other substances with which he comes in contact. Hypersensitivity reactions are side reactions of this primarily protective mechanism but as they involve tissue damage it is doubtful whether they are ever in themselves beneficial.

After the first exposure to a foreign substance these reactions of immunity or hypersensitivity are not observable. Changes however do take place in the organism resulting in a state of 'changed reactivity' for which von Pirquet (2) coined the term allergy from allos, indicating a deviation from normal and ergon (work). In his admirably lucid paper, von Pirquet indicates clearly that the term immunity should be 'restricted to those processes in which the introduction of the foreign substance into the organism causes no clinically evident reaction, where, therefore, complete insensitivity exists'. Hypersensitivity, in contrast, is always accompanied by obvious clinical signs.

Von Pirquet further described those substances which induced allergy as

allergens. These 'comprise, besides the antigens proper the many protein substances which lead to no production of antibodies but to supersensitivity'. Not only are 'the agents of infectious diseases which are followed by immunity' allergens but also the poison of insects in so far as the stings are followed by hypo- or hypersensitivity, pollens causing hay fever, and simple chemical substances capable of causing contact dermatitis.

Coombs (3) restricts the use of the terms allergy and allergic to the cellular and humoral changes underlying the chemical reactions of immunity and hypersensitivity. The initial allergic response to an allergen is the establishment of the allergic state which, as it were, primes the organism so that further exposure to the allergen produces an allergic *reaction* resulting in clinical immunity or hypersensitivity. As will be readily appreciated this follows von Piquet's original definition and is the usage adopted by most immunologists. However, many clinicians and dermatologists use the term allergy to describe reactions of hypersensitivity exclusively.

The type of immune or hypersensitive reaction produced depends to some extent on the nature and dose of the foreign antigen or allergen and also on the route and method of its introduction. Those allergic reactions which, because they are accompanied by cellular damage, may form the basis of clinical hypersensitivity have been divided into four types by Coombs and Gell (4). The essential features of these various allergic reactions are given in *Table I.* It will be seen that Types I, II and III are all dependent on antibody production, while Type IV is mediated by actively allergized cells. This major difference and the later appearance of Type IV reactions separates the four allergic reactions into two broad categories, immediate or humoral hypersensitivity and cell-mediated or delayed hypersensitivity (5).

Type I. Immediate hypersensitivity

Prausnitz and Küstner (6), both of whom suffered from this form of hypersensitivity, demonstrated that a serum factor was responsible for producing the typical symptoms in conjunction with the appropriate allergen. Since this factor did not produce precipitates when mixed with the allergen *in vitro* it could not be detected by the usual laboratory tests for antibodies and was, therefore, given the non-committal name of reagin (7).

Individuals prone to infantile eczema, asthma or hay fever, and who readily produce reagins, were named atopic by Coca and Grove in 1925 (7). The nature of reagin was elucidated after extensive research by Ishizaka and Ishizaka (8–15). They found it was a globulin, now named IgE, with various

Table I Allergic reactions producing hypersensitivity

Type of reaction	I Anaphylactic	II Cytotoxic	III Arthus-type associated with toxic complexes	IV Delayed cell mediated
Time interval between exposure of sensitized animal to allergen and symptoms of hypersensitivity	'Immediate'. As little as 10–20 min	Not as immediate as Type I, but within 24–48 h	4–8 h	Delayed 24–72 h
Principal antibody or cell mediating reaction	Humoral antibody, *non* precipitating. Binds strongly to tissues. Inactivated at 56°C. Immunoglobulin IgE, in human	Humoral antibody. Precipitin. Not inactivated at 56°C Immunoglobulin IgA, IgG. May activate complement	Humoral antibody. Precipitin. Not inactivated at 56°C. Immunoglobulin IgA, IgM or IgG. Activates complement	No humoral antibody. Initiated by modified (allergenized) lymphocytes
Mechanism of allergic reaction	Allergen reacts with antibody sensitized cells (e.g. basophils) which produce histamine and other active substances	Antibody reacts with antigenic component of a cell membrane or with antigen or hapten (usually a drug) associated with the membrane	Antigen reacts with antibody to form micro-precipates in tissue spaces or circulating complexes in the blood. Complement activated and chain reaction results releasing tissue damaging substances	The actively allergized lymphocytes react with allergen locally. Pharmacological agents are released which act on macrophages and other cells
Tissue reactions: Local	'Wheal and flare' in skin and mucous membranes. Increase in vascular permeability leading to oedema	Disintegration of cell with which antibody reacts	Arthus reaction. Local vascular thrombosis, polymorph infiltration, oedema and necrosis	Local erythema and induration in skin or mucous membrane. Infiltration of mononuclear cells (polymorphs in some species)
Systemic	Contraction of smooth muscle especially bronchial	Dependent on cell primarily involved, e.g. haemolysis to anaemia if erythrocyte is the target cell	'Delayed serum sickness' (24 h to 4 days). Late asthmatic reactions (4–6 h)	
Clinical states associated with the hypersensitivity	Anaphylaxis, asthma, pulmonary oedema, generalized urticaria, hay fever	Haemolytic disease of newborn. Purpura associated with sedormid. Haemolytic anaemia, associated with phenacetin or para amino salicylate	Drug sensitivity, e.g. penicillin and sulphonamides. Disease of the lungs due to fungi and organic dusts. Possibly nephritis, polyarteritis, rheumatoid arthritis. Disseminated lupus erythematosus	Contact (allergic) dermatitis. Vaccinia. Tubercle in tuberculosis. Insect bites. Allograft rejection. Rejection of neoplastic cells

characteristics differentiating it from the known globulins, IgA,–M,–G or –D. The discovery of a biologically similar new myeloma protein (16–19) and the corresponding normal protein (20) complemented their work and during the last few years there has been a considerable advance in the understanding of the immunological basis of Type I hypersensitivity (21).

Although atopic individuals have higher IgE levels than normal persons owing to the facility with which they form IgE antibodies to a variety of allergens, non-atopic individuals can form IgE antibodies in response to some antigens, in particular, those of ascaris and other intestinal parasites.

Antibodies very similar biologically to reagins have been produced experimentally in several species (22). Hypersensitivity to ragweed associated with an antibody in all ways comparable to human IgE occurs naturally in dogs (23, 24) and monkeys infected with ascaris produce a non-precipitating heat labile skin sensitizing antibody of the same type (25).

Type II. Cell binding

Comparatively simple chemicals (haptens) or bacterial antigens can form associations with cell membranes, and initiate the production of antibodies. In the subsequent antibody–antigen reaction the cell membrane is destroyed and the cell disintegrates. Examples of drugs associated with this type of hypersensitivity are:

Apronalide (Sedormid) which is absorbed onto platelets whose subsequent destruction results in diminution of blood coagulation and purpura (26).

Phenacetin which forms a loose association with red cell membranes, the resulting destruction of which causes haemolytic anaemia (27).

Type III. Arthus (28) *reaction and immune complex disease*

The combination of antigen with antibody to form microprecipitates forms the basis of this type of reaction. The accumulation of these antigen–antibody complexes in tissue spaces, and particularly in and around small blood vessels causes damage to cells secondarily.

The typical example of this type of hypersensitivity is classical serum sickness following repeated therapeutic use of antibacterial or antitoxic sera derived from animals.

Other examples are:–

Soluble antigen–antibody complexes circulating in the blood vessels at the joints, renal glomeruli, skin or heart and cause cellular injury resulting in arthritis, nephritis, polyarteritis or carditis.

Inhalation of such allergens as spores of micropolyspora faeni in non-atopic subjects invokes the formation of precipitins. These precipitins mediate local Type III Arthus reactions especially in the alveoli (29). Pepys (30) has suggested the term extrinsic allergic alveolitis for this particular disease, known as Farmer's lung, and other similar diseases due to inhalation of organic dusts.

The intradermal injection of antigen into an animal with a corresponding precipitating antibody will produce an intradermal reaction of oedema, local vascular thrombosis, polymorphonuclear infiltration, haemorrhage and necrosis 4–8 h later.

Type IV. Delayed cellular hypersensitivity

Types I, II and III hypersensitivity are mediated by a humoral, circulating antibody, and can be transferred to a non-affected individual by injections of the patient's serum. No humoral antibody has been isolated in Type IV, and it is mediated by sensitized lymphocytes. Only the transfer of such cells will passively sensitize another host (31–33).

These actively sensitized lymphocytes will react *in vitro* or *in vivo* with antigen and as a result pharmacologically active agents are released which promote mitosis in normal lymphocytes (34–36), immobilize macrophages (37–43), cause cell destruction (44, 45) and produce an inflammatory reaction in the skin (46–48).

This type of reaction is involved in the rejection of tissue grafts from other individuals and possibly in the destruction of mutant neoplastic cells. Normal skin which has been antigenically modified by conjugation with simple chemical haptens is similarly rejected in contact dermatitis.

The same reaction with extrinsic antigens gives rise to lesions typical of delayed hypersensitivity seen in relation to infection with viruses (e.g. vaccinia), bacteria, fungi, yeasts or protozoa. Similar reactions are produced by insect bites or by the injection of any heterologous protein or hapten–protein conjugate into a suitably sensitized individual.

Inter-relationship of the various types of hypersensitivity

The actual type of hypersensitivity developed would appear in large measure to depend on the following factors.

The nature and dose of the allergen involved. Benzyl penicillin (Penicillin G) in a skin ointment or cream can give rise to contact dermatitis but if injected intramuscularly, especially into atopic patients, can give rise to skin sensitizing antibodies and Type I hypersensitivity. High doses of the same

drug, especially if given intravenously, may be followed by the production of IgM antibodies and symptoms suggestive of immune complex disease (Type III hypersensitivity) (49).

Some allergens such as pollens are particularly associated with reaginic antibody formation in genetically susceptible atopic subjects, whereas some drugs taken systemically tend to be usually associated with Type II hypersensitivity.

The way in which the organism comes in contact with the allergen. Experimentally, much work has been done on the association between delayed type dermal hypersensitivity and the development of circulating, precipitating antibodies and Arthus type local reactions to the same allergens (50–52). Salvin and Smith (53, 54) and later Leskowitz (55) have suggested that delayed hypersensitivity may be a preparation for antibody synthesis and Type III hypersensitivity. Salvin and Smith found that minute doses of the injected allergen could be followed by Type IV hypersensitivity. Leskowitz and Waksman (56) emphasized the importance of the site of injection. Injections of a given allergen intramuscularly or subcutaneously produced high levels of circulating antibody in guinea-pigs, whereas injections into the toe pad and peritoneal cavity were less effective and intravenous and intradermal least effective. Delayed type hypersensitivity was by contrast most readily produced by injections intradermally or into the toe pad. Intramuscular, subcutaneous and intraperitoneal injection were less effective and intravenous injection completely ineffective.

Similarly atopic individuals exposed to airborne allergens are liable to Type I hypersensitivity reactions. Intramuscular injection of the same allergens will produce precipitating antibodies in the same individuals.

The presence of circulating antibody would not appear to have any effect on the local delayed hypersensitivity reaction. Guinea-pigs with delayed type hypersensitivity to sheep erythrocytes but with no circulating antibodies had their skin infiltrated with an antibody conferring passive cutaneous anaphylaxis to sheep erythrocytes before receiving an intradermal challenge of the allergen. The ensuing delayed type dermal reaction was not enhanced in comparison to reactions in guinea-pigs not receiving antibody (57).

Genetic factors. Atopic individuals do not appear to be more prone than normal individuals to develop delayed type hypersensitivity to chemicals and other allergens with which they come in contact (58–61) although they may be more liable to develop Type I hypersensitivity to the same substances. In such reports as exist of increased incidence of contact dermatitis in

sufferers from atopic eczema, it seems probable that this increase can be entirely explained by the increased use of medicament containing creams by such patients, coupled with the fact that their eczematous skin is less protected from exogenous allergens than is normal skin.

In reverse, it does appear that some individuals are genetically predisposed to develop delayed type hypersensitivity (62, 63). They again do not appear to be any more prone than normal persons to develop other types of hypersensitivity.

Types of hypersensitivity associated with the use of cosmetics

As already noted, the type of hypersensitivity developing is associated with the way in which contact is established with the allergen. In general, Types I, II and III follow exposure to antigens which are inhaled, ingested or injected. The immediate type of skin sensitivity (Type I) associated with reagins is a by-product of the systemic hypersensitivity, but the eczema to which atopic individuals are prone does not appear to be directly caused by skin contact with allergens to which the sufferer is sensitive, even though intradermal injection of these causes a positive skin reaction (64). In contrast asthma and hay fever in atopic individuals do follow inhalation, ingestion or injection of the sensitizing allergen.

From their very nature, some cosmetics are likely to be involved in these types of hypersensitivity in the following circumstances:

1. Toothpastes, mouth washes and to some extent lipsticks are ingested and could theoretically be implicated in Type I, II or III reactions if they contain appropriate allergens.

2. Many cosmetics in liquid or powder form are now available as aerosols and are especially liable to be inhaled. Examples are hair sprays, perfumes, dry shampoos, nail varnish driers, antiperspirants, deodorants, hand and skin creams, foam bath preparations, bathing and sun tan oils.

Volatile components of liquid preparations will also be inhaled in smaller quantities from the lotion or perfume *in situ*.

If the appropriate allergens are present, Type I immediate sensitivity reactions may follow. Sidi *et al* (65) reported 17 cases of hypersensitivity to sericine (fibroin) in a new hairdressing product. Fibroin is a nitrogenous substance obtained from silk worm cocoons and is the base for the isolation of serine. Although a contact dermatitis was diagnosed in some patients, 14 presented with asthma and/or urticaria, symptoms characteristic of Type I hypersensitivity. The asthma followed inhalation of the product while using

it on clients (14 hairdressers), during and after application (one client) or while working in an establishment where the product was manufactured or used (two employees). The latter had had no direct contact with the sericine. Typical wheal and flare reactions were obtained on skin testing the patients. The authors refer to other examples of hypersensitivity to sericine in the silk industry and note that these, as well as those reported in their paper, were not confined to atopic individuals (65).

In other reports of asthmatic attacks due to sensitization by cosmetic products the sufferers almost invariably had a history suggesting that they were atopic and usually gave positive skin tests to a number of other common allergens besides those implicated in their current attacks. Thus Gelfand (66) reported 14 patients with asthma, rhinitis or conjunctivitis who were employees and patrons of beauty culture salons or home users of similar preparations. All had a history of atopy. One patient suffered as a result of his wife's devotion to beauty culture and had positive intradermal skin tests to ethylene diamine, ammonium, thioglycolate, monoethanolamine and hexamethylenetetramine. All the other patients also gave positive skin reactions to the first three chemicals. Cold wave solutions and nail spray or lacquer were the products particularly implicated.

Key (67) cited a number of chemicals which may produce Type I or Type IV hypersensitivity. Symptoms of Type I were usually asthma or hay fever but instances of urticaria were also reported. These allergens included the following chemicals which might possibly be present in cosmetics:

Aliphatic polyamines	Paraphenylene diamine
Formaldehyde	Phthalic anydride
Karaya gum	Pyrethrins

Exogenous allergic alveolitis, where delayed asthmatic attacks follow exposure to the allergen, is believed by Pepys (68) to be a Type 3 Arthus type reaction to inhaled allergens. Although no proof is available it seems at least possible that the examples of granulomatous lung lesions in the literature (69–72) possibly associated with the use of hair sprays could be due to hypersensitivity of the Type III variety such as has been implicated with Farmer's lung and other similar lung lesions. The polyvinyl pyrrolidone (PVP), shellac or other resin employed in the spray might only be a vehicle for some other antigen. In only one paper (73) was any search for antibodies in the patient with so called thesaurosis reported.

Actual data on the humoral types of allergic reactions in relation to specific cosmetics is relatively sparse and there is no question that, to date at least, the chief hypersensitivity that seems likely to follow the use of cos-

metics is that of the delayed cellular type manifesting as contact dermatitis since most cosmetics other than those listed in 1 and 2 above are designed for direct application to the skin and its appendages. Ingestion and inhalation are only minimal hazards with such products.

Many products used in cosmetics are not peculiar to them and are used in other products in the home, or office or factory. The possibility of becoming sensitized to azo dyes, for example, is much higher when actually working in a dye factory than in a beauty salon. Nevertheless, sensitization induced in the factory or elsewhere will be elicited by the use of the same ingredient in a cosmetic preparation and, moreover, may be induced by very small amounts. Several groups of chemicals produce cross-sensitization reactions. That is, if an individual is sensitized to one member of the group exposure to another member may produce the symptoms of the particular sensitivity involved. An example of such a cross-sensitizing group is the so called '*para*' group which includes:

p-phenylene diamine	Aniline
p-aminoazobenzene	Nitrophenols
p-aminodiphenylamine	Sulphonamides

Cosmetics as a source of allergens

Complete antigens are comparatively rare in cosmetics but can be included in the form of lipoproteins, amino acids (methionine etc.) and various egg products. This class of allergens is in general more often associated with a Type I, II or III hypersensitivity than with a contact dermatitis. Most allergens in cosmetics are 'simple' chemicals which in this context simply means haptens or compounds which are antigenic only after association with protein.

Potential allergens, mostly haptens, which are, have been or might be incorporated in various cosmetics are listed in *Table II*. The products in which they are most likely to be incorporated are shown in *Table III*. Evidence produced by one dermatologist, that a given chemical has been or might be implicated in reactions of hypersensitivity, is by no means always accepted by other investigators. Where possible, references have been given to divergent opinions on the status of substances in the table.

Detecting the chemical responsible for hypersensitivity reactions is not easy and many years may elapse before sensitizers in a product are identified and proven cases of contact dermatitis or asthma following their use are

Table II Potential contact allergens in cosmetics

Category	Compound	References	Comments
A. Ointments and emulsions Vehicles	Cetyl and Stearyl alcohols	74, 75	
	Ethylenediamine* HCl	75, 86, 66	*Type I also
	Eucerin	76	
	Lanette wax	83	
	Lanolin	75–81, 219	
	Oleyl alcohol	82, 83	
	Polyethylene glycol	84	
	Polyethylene glycolmonostearate	75	
	Propylene glycol	75, 84–86	
	Triethanolamine*	75, 87–90	
	Tween 80	86	One example of positive patch test
B. Preservatives	Bithionol	91, 92	Photo-allergen
(a) Antimicrobials	Cetrimide (Cetyltrimethylammonium bromide)	93, 94	
	Chlorocresol	74	
	Chlorxylenol	74, 94	
	Dichlorophene	95–97	
	Formaldehyde*	66, 79, 98–101	*Type I also
	Halogenated hydroxy-quinolines	102	
	Halogenated salicylanilides	86, 102, 115	Photo-allergens
	Hexachlorophene	83, 221	
	Mercury bichloride	86, 98	
	Neomycin	86, 103	
	Parabens (methyl, ethyl, propyl and butyl esters of *p*-hydroxybenzoic acid)	74, 75, 104, 105	
	Penicillin	86	
	Phenylmercuric acetate	74, 75	
	Quaternary ammonium compounds (cetrimide—above), e.g. benzalkonium chloride	220, 83 110	
	Dequaline chloride (decamethylenebis[4-aminoquinaldinium chloride])	95	
	Sodium ethyl-mercurisalicylate (merthiolate)	75, 83, 94, 98	
	Sorbic acid	74, 75, 95, 111, 112	
	Thiuram sulphides	102	
	Triazines	102	
(b) Antimycotics	Pyrethrum*	66, 83	*Type I also
	Phenylmercuric borate	83, 95	

Table II continued

Category	Compound	References	Comments
C. Antioxidants	Butylated hydroxyanisole	83	
	Diaminodiphenylmethane	86	
	Hydroquinones	113, 114	See F
	N-phenylcyclohexylamine	83	
	Propyl, octyl and dodecyl gallates	83	
	Tocopherol (Vitamin E)	83	
D. Colouring agents	Azo dyes	114, 115	See below
	Aminoazotoluene	79	
	Bismarck brown	113	
	Carmine (aluminium lake cochineal)	116	
	Cochineal (Anthraquinone)	115	
	D and C Orange No. 17 (Permanent Orange)	116	
	F D and C Red No. 2 (Amaranth)	113, 116	
	F D and C Red No. 3 (Erythrosine)	113	
	F D and C Red No. 4	113	
	D and C Red No. 17 (Sudan III)	113	
	D and C Red No. 19 (Rhodamine)	116, 117	
	D and C Red No. 31	117	
	D and C Red No. 36 (Permaton red)	116	
	Dispersol Fast Yellow G (colour index (1956) 11855)	116	
	Eosin (and other halogenated fluoresceins)	116, 117	
	o-nitro-*p*-phenylenediamine	117	
	p-Aminodiphenylamine	121	
	p-Phenylenediamine*	79, 112, 118, 119	*Type I also
	p-Toluenediamine	120–123, 124	
	Tolu safranin	114	
E. Perfumes and flavourings including modifiers, fixatives for perfumes	Almond oil	113	
	Anise	127	
	Cade oil	129	
	Catechols (see below)	127	
	Cinnamon	86, 113, 125–127	
	Clove oil	86, 127	
	Eugenol	127	
	Ginger oil	113	
	Heliotrope	115	
	Citronella	115	
	Ionene (Violet)	115	

Table II continued

Category	Compound	References	Comments
	Laurel oil	102	
	Methylheptine Carbonate (Jasmine)	115	
	Orange oil	86	
	Peppermint	127	
	Terpenes	115, 127	
	Spearmint	127	
	Vanilla (Vanillin)	113, 125, 127	
Fixatives	Balsam of Peru (Coniferyl benzoate)	86, 123, 125, 128, 129	Inter-related
	Balsam of Spruce	125	
	Balsam of Tolu	125	'Group sensitizers'
	Benzoin Styrax	125, 127	
	Benzoic acid	125	
	Benzyl Benzoate	86, 125	
	Benzyl salicylate	128, 129	
F. Bleaching agent (skin)	Monobenzyl ether of hydroquinone	127, 133	
	Ammoniated mercury and other mercury compounds	82, 86, 96	
G. Artificial tanning and sunscreen agents	Dihydroxyacetone	115, 127	
	p-Amino-benzoic acid esters	83, 127	Photo-allergens
	Digalloyl trioleate	102	Photo-allergen
H. *Resins* Natural and artificial plasticizers, plastics and constituents	Arylsulphonamide formaldehyde resin	117	
	Benzoin	127	
	Glycidyl ethers of Bisphenol A type	83, 102, 127	Cross react with Diethyl stilboestrol
	Colophony (Rosin)	79, 86, 137	
	Ethylenediamine (Stabilizer for lacquers)	66, 134–136	See A
	Formaldeyde*	141, 142	See B
	Hexamethylenetetramine*	66, 127, 132	
	Karaya gum*	67	
	Linseed oil	139	
	Methyl methacrylate (acrylic plastic)	143, 144	
	Monoethanolamine* (Hair set lotion)	66	*Type I
	Phthalic anhydrides*	66, 101, 138	*Type I also
	Triethylenetetramine (hardener)	83, 127	
	Silicones	145	
I. Waving lotions and other hair lotions	Quinone	79, 83, 127	
	Resorcinol	83, 86, 127, 220	
	Sericine (fibroin)*	65	*Type I also

Table II continued

Category	Compound	References	Comments
	Thioglycerol	130, 131, 146, 216	
	Thioglycollates* (principally ammonium)	66, 123, 146, 147*	Type I also
	Mercaptans (other)	132, 216	
J. Miscellaneous	Zirconium (deodorants)	148–150	Atypical granulomatous lesion
	Guanine (2-amino-6-hydroxypurine) (Frosting in nail lacquers)	151	
	Nickel (an impurity in detergents)	109, 124, 152	
	Chromates (in detergents and Bleaches—Eau de Javelle)	123, 153	
	Azulene (anti-irritant)	117	
	Coal tar (soaps etc.)	79, 86, 140, 220	Photo toxic agents
	Quillaja (in some coal tar preparations)	127	
	Diethylstilboestrol (hormone creams)	102, 127	Cross reacts with Bisphenol A
	Antihistamines	154, 156–158, 159	

recorded in the literature. This is especially true of weak allergens. *Para*-phenylenediamine and similar strong sensitizers are usually recognized early because a high percentage of individuals react to them.

A fairly characteristic pattern is observable with weaker allergens. Initially they appear innocuous, then after a varying time interval, instances of allergic reactions are recorded among factory employees engaged in processing the relevant raw materials or manufacturing products containing the allergen. Later individuals using the product frequently report with similar reactions and last of all ordinary consumers may be affected. With a cosmetic product, hairdressers and workers in beauty salons are likely to become sensitized to a new product before members of the general public. Products containing the allergen in higher concentrations are also more likely to be incriminated before those in which it is present in small amounts. Methyl, ethyl, propyl and butyl *p*-hydroxybenzoates may be present in medicaments in a much higher percentage (up to 5% in fungicidal ointments) than they are in cosmetics (below 5%) and the existence of contact dermatitis following their use in cosmetics *only* is disputed by some dermatologists (95, 111). Fisher (75), however, considers that high concentrations are not

Table III
Allergens possibly associated with various types of cosmetics

	Group 1	Group 2	Group 3
Method of application	Topical to skin or appendages. Unlikely to be inhaled or ingested	To teeth, mouth, lips. May be ingested	Applied to skin or appendages by spray. May be inhaled
Type of sensitivity theoretically possible	Type IV delayed	Type IV* Type I (Types II & III)	Type IV Type I (Type III)
Type of product	(a) Face creams, powder, rouge, hand and body lotions Table II. A, B, C, D, E, G F, J in special creams	(a) Toothpaste,* Mouth washes* B,D,E,	(a) Hair sprays, lacquers, lotions, conditioners, etc. A, B, C, (D), E, H, I.
	(b) Nail lacquer. H, J. (Guanine) see Group 3.	(b) Lipsticks A, D.	(b) Shaving lotions B, E, J.
	(c) Hair-dyes, creams, lotions, shampoos, conditioners, etc. see Group 3.	(c) Soaps, bath essences etc. (especially children) A, B, C, D, E, J.	(c) Perfumes E.
	(d) Depilatories I. Thioglycollates H. Waxes, adhesives.	*Contact stomatitis of buccal mucous membranes equivalent to contact dermatitis can occur (100,127)	(d) Deodorants and antiperspirants B, E, J.
	(e) Shaving lotions see Group 3 A, B, C, E, J.		(e) Nail varnish, etc. H, J.
	(f) Deodorants A, B, E, J (Zirconium) See group 3 Includes vaginal deodorants N.B. Contact lesions of mucous membranes may occur.		(f) Bath preparations A, B, C, D, E, J. (g) Sun tan oils A, G.

necessary and repeated applications of the low concentrations of parabens in cosmetics and dentifrices appear to be sufficient for sensitization to occur.

Parabens illustrate a further difficulty in the detection of sensitizers. Although products containing only 0.05% parabens may produce contact dermatitis in a sensitized individual with regular use, patch testing at a similar concentration may be negative, and concentrations of 3–5% are necessary to produce a positive patch test reaction (75, 111).

It should be emphasized that once hypersensitivity to any chemical has been established reactions are liable to follow exposure to even very small amounts. A patient once sensitized to parabens cannot use a paraben-containing cosmetic. Similarly, contact with any chemical that cross-reacts with the original allergen may cause contact dermatitis.

Seasonal variations in the incidence of positive patch tests to various substances have been reported—by Hjorth (161), and Calnan (162) has reviewed at length the influence of both macro- and microclimate on the incidence of contact dermatitis and patch test reactions. This introduces other variables in assessing the hazard of a given product.

Chemical nature of contact sensitizers

Landsteiner and Jacobs (163) first indicated that the chemical structure of compounds influenced their skin sensitizing potential. In particular the position of Cl or NO_2 groups determines the ease with which these chemicals can become attached to proteins of skin and other tissues. This altered protein is 'foreign' to the host animal who reacts accordingly. Some chemicals such as *p*-phenylenediamine are themselves unreactive but are readily oxidized or otherwise altered to form more reactive metabolites (164). Eisen and Tabacknick (165) demonstrated the importance of conjugation with protein in the basal layers of the skin in the elicitation of contact dermatitis in the guinea-pig. Generalized sensitization of the skin in these animals can follow initial contact of the allergen with vaginal, uterine or colonic mucous membrane. In reverse, the mucosa of guinea-pigs sensitized by skin contact only will react positively to patch test (166). Conjugation of the allergen with skin keratin is thus not a pre-requisite of contact dermatitis. The mechanism of contact sensitization is well reviewed by Calnan (167) and Schild (168).

Tests for allergens

There is abundant data on various types of tests for use clinically, experimentally and predictively. Some tests are primarily designed for detecting sensitization in patients or experimental animals and these are obviously of prime importance clinically. Predictive testing which endeavours to detect potential allergens involves the use of similar techniques and principles so both types of tests will be considered.

Tests for sensitization with (or without) clinical signs and symptoms

Presented with a patient suffering from what appears to be a clinical hypersensitivity, various tests can be applied. Association between positive tests for a given allergen and the symptoms is usually assumed. In practice the sensitization demonstrated may be incidental to the symptoms. Withdrawal of the allergen followed by loss of symptoms strengthens the conviction that it is the causative agent. Provocative tests followed by re-occurrence of the symptoms are almost completely conclusive.

Some or all of the same tests may be positive in individuals who have no clinical symptoms or signs but who have been exposed to and sensitized by the test allergen and are, therefore, in an 'allergic state'.

Tests for hypersensitivity of Types I, III, and IV are summarized in *Table IV*. Type II hypersensitivity is not included as being largely irrelevant in the present context. In general antibody–antigen tests listed in section E of *Table IV* for Type III are also appropriate to Type II.

Intracutaneous skin tests and inhalation tests in Type I hypersensitization have resulted in death. Adrenaline must be available for immediate intravenous use if an extreme reaction follows testing. Very low concentrations of the suspected allergen should be used first and subsequent tests using higher concentration used cautiously. The prick test (169, 170) is reliable and much safer than an intracutaneous test. It consists of introducing the point of a No. 26 gauge needle into the epidermis through a drop of test allergen. It is estimated that 0.3 nl of the liquid are introduced compared with 0.01 ml by the usual intracutaneous test (171).

Patch testing in contrast is a safe technique, discomfort being the main hazard. There have been many modifications of the original method devised by Jadassohn and later Bloch (172), but the general principle remains the application of the allergen to the forearm or back on some inert material covered by an occlusive dressing held in place by adhesive tape.

Table IV
Tests for clinical hypersensitivity in man

Test	Type I	Type III	Type IV
A. Skin tests			
(1) Method	(a) Prick (168. 169) (b) Scratch (c) Intracutaneous	As for Type I	(a) As for Type I when testing for sensitization to various bacteria, viruses, fungi or their products (b) Patch test for contact dermatitis or skin sensitization
(2) Result Positive reaction	Urticarial *wheal* Erythematous *flare*	Area of raised, ill-defined oedema (Arthus reaction)	(a) Indurated nodule (b) Erythema—papules and vesicles
(3) Time interval to positive reaction	10–20 min. Fades within $1\frac{1}{2}$ h	3–4 h. Maximal 7–8 h. Subsides within 24 h	24–48 h or longer May take several days to disappear completely
B. Provocative inhalation test (173)	(a) Inhalation of powder, spray from atomizer etc. Low concentrations initially (b) 0.05 ml of allergen in solution (10^{-11}g/ml initial concentration) placed in nostril	As for Type I	Not applicable
Positive reaction	Sneezing, wheezing, asthmatic reaction	Same	
Time interval	Immediate Resolving in 1–2 h.	4–6 h (174)	
C. Prausnitz Küstner test (6, 178)	Injection of patients. Serum intracutaneously into non-sensitized subject, followed by i.c. injection of allergen results in typical wheal and flare reaction	Not usually used as antibodies are detectable by laboratory techniques. If used Arthus type reaction follows	Negative Typical reaction can only be transferred by sensitized lymphocytes or transfer factor isolated from them
D. Passive cutaneous anaphylaxis in monkeys (175)	Intracutaneous injection of patient's serum followed by intravenous injection of allergen	As for C, Arthus type reaction follows if test is applied	Negative

Table IV continued

Test	Type I	Type III	Type IV
D. Results	Erythema, at site of serum injection. Trypan blue intravenously is extravasated at site. Immediate	Arthus reaction 4–6 h	Negative
In vitro tests			
E. Antibody–antigen test	Antibody does not precipitate antigen	1. Precipitation test 2. Agglutination tests. Direct and indirect 3. Haemolytic test 4. Gel diffusion techniques (176)	No circulating antibody detectable
F. Reaction of actively allergized cells			Experimental systems only (a) Lymphocytes from patient's blood undergo 'blast' transformation in culture with the allergen (182, 183) (b) Inhibition of macrophage migration in culture with antigen (40, 184) (c) Lymphocyte-cytotoxicity (185)
G. Reactions of passively allergized cells	(a) Double layer leucocyte agglutination test (DLLA) (178) (b) Histamine release from passively sensitized leucocytes (177, 179) (c) Basophil degranulation test (180, 181)		
H. Other laboratory or experimental tests	(a) Schultz-Dale with *in vitro* passively sensitized monkey ileum (186), human appendix (187) (b) Measurement of histamine release from passively sensitized human (178) or monkey lung (188) (c) Skin window technique. Demonstration of eosinophilia (189, 190)		

Circulating precipitating antibody, present in Type III hypersensitivity, may be detected by a variety of *in vitro* tests all of which involve antigen antibody reactions with a visible component, e.g. precipitation in a tube or gel. Such simple direct tests are not possible for Type I or IV since no circulating antibody can be detected in the latter and circulating Type I antibody is not a precipitin. This has led to a multiplicity of *in vitro* and *in vivo* tests of varying reliability and practicability. Their possible value in predictive testing will be discussed in detail later.

Predictive and laboratory tests for allergens

Obviously these tests are of prime interest to the cosmetic manufacturer, but clinical tests are not irrelevant as much work has been done on standardizing and evaluating the technique of clinical patch testing especially by Scandinavian dermatologists (191–199) which is of importance in the predictive tests for allergens.

Routine tests for allergens, either as separate ingredients or in products, are performed on guinea-pigs or in man. In all tests the assumption made primarily is that any allergen present will induce delayed type sensitivity. This view is justified by contact dermatitis being the prime reason of complaint in this particular area of hazard in the use of cosmetics. However, no attempt is apparently made to test for, or consider testing for, the ability of cosmetic ingredients to induce Type I and still less Type III allergy. That they can at least elicit the symptoms of Type I allergy and may be implicated in Type III allergy has already been discussed. It seems, therefore, reasonable that some consideration should be given to testing at least those ingredients that may be inhaled.

TESTS FOR CONTACT SENSITIZERS

These will be considered first since contact dermatitis from the use of cosmetics is to date a greater reported hazard than other types of sensitization.

Animal testing

This is a necessary preliminary to human testing but cannot be a substitute for it. Different species vary in both the degree and the nature of their reactions to different allergens and none can be completely equated

with man. Bloch (200) and Landsteiner and Jacobs (201) in their early experiments on sensitization of animals with chemicals used the guinea-pig and succeeded in obtaining reproducible results. This animal has remained the main experimental model and there seems little reason to change to another species, unless one can be shown to be as sensitive, as easily obtainable and as cheap. Rostenberg and Haeberlin (202) in a survey of the ability of other species to develop eczematous sensitization to simple chemicals concluded that chickens, ferrets, monkeys and pigs had shown positive evidence of such sensitization. There was only questionable evidence in dogs, rabbits and white mice and none at all in cats, hamsters or rats. They suggest that the observed variability may be due to the inability of the test chemicals to unite with protein of the non-reacting test animals. This would accord with the generally accepted view that to be allergenic a hapten or its metabolite must be able to combine with host protein.

Apart from actual variations in experimental technique, factors which can influence the response of guinea-pigs to potential contact sensitizers are:

Heredity

Chase (203) showed that susceptibility is influenced by genetic factors and was able to breed susceptible and resistant strains. Munoz (204) reported on the different responses of Hartley and Strain 13 guinea-pigs to various antigens injected by different routes. Genetic differences may extend to the ability or inability to respond to various hapten conjugates (205) or inorganic metal compounds (206).

Time of year and temperature

There is a general consensus of opinion that it is easier to sensitize a given strain of guinea-pig in winter than in summer (207–209). Rockwell's experiments (210) seem to confirm the correlation between low temperature and increased reactivity but the physiological basis of such observations is not clear.

Ascorbic acid

An inadequate intake of ascorbic acid is associated with a lowered response to sensitization (211).

Prior feeding of the test allergen

Battisto and Chase (212, 213) have shown that guinea-pigs fed a given allergen may remain unresponsive to delayed hypersensitization by the

same allergen for as long as 10 months while remaining responsive to unrelated chemicals.

The standard tests approved by the Food and Drug Administration of the United States of America are based on those devised by Landsteiner and elaborated by Draize (214). Various modifications exist and the principal ones are summarized in *Table V.*

The main requirements of an animal test for sensitization are that it should be easily performed using a minimum of complex apparatus, be reliable and reproducible and give accurate information about the sensitizing capacity of a substance which can be applied to man.

It is generally assumed that a substance which produces sensitization in the guinea-pig will also do so in man (218, 219). However, the guinea-pig is not as susceptible to sensitization and hence the repeated search for modifications which will increase the value of existing animal tests. Earlier tests were satisfactory for strong allergens only. Voss (216) devised his test for screening mercaptans which might be of use in hair-waving lotions. He increased the concentration of test substance to the highest possible without producing irritation—an obvious advance on an overall use of a standard 0.1% solution. Of the 19 mercaptans tested in man and the guinea-pig, 8 sensitized both species and 11 sensitized man only. None sensitized the guinea-pig and failed to sensitize humans. Buehler (217) with similar aims to those of Voss, used a closed-patch technique based on the methods used for inducing sensitization in man. The closed patch, producing as it does an area of moist skin with increased permeability, increases the sensitization rate. Comparable degrees of sensitization were produced using 0.05% dinitrochlorbenzene under a closed patch, 1% topically and 0.25% intradermally, demonstrating the greater sensitivity of the closed-patch technique. In routine testing the test substance was again used at a concentration just below the threshold of primary irritation. Comparisons of the Landsteiner and closed-patch techniques were made with six known contact sensitizers. Only two (potassium chromate and formalin) were detected by the former, while some at least of the guinea-pigs tested by closed patch became sensitized to all six. In particular tetrachlorosalicylanilide, thioglycerol and *p*-phenylene diamine hydrochloride failed to produce sensitization in any of 10 guinea-pigs tested by the Landsteiner method while using closed patch 8/10, 6/10 and 10/10 guinea-pigs respectively were sensitized. Buehler failed to sensitize guinea-pigs with inorganic salts of mercury, nickel and cobalt.

Stevens (218) again used topical applications of the test agent, painting it on to the ear in an appropriate vehicle.

In this site the guinea-pig apparently does not interfere with the test substance. A distinctive feature of Stevens' method is that the whole test is completed in 8 days. This period was adequate for producing sensitization to a wide range of chemicals tested.

The importance of the vehicle used in the test is stressed. Using dinitrochlorbenzene (DNCB) as the test agent, animals were successfully sensitized with all the vehicles used, but the degree of sensitization varied considerably. No reaction in 36 guinea-pigs scored more than 'trace' or '±' with acetone, glycerine and ethanol as vehicles and only 1/36 with propylene glycol. Scores with dinonyl phthalate (5/36), olive oil (6/36) and liquid paraffin (5/36) were rather higher but dimethyl formamide gave '+' results with 11/36 animals and *Tween* 80 produced the most marked erythema, 25/36 animals scoring '+' or '++' reactions. *Tween* 80 also produced some erythema in controls.

Stevens, pointing out that skin sensitization is almost always a 'percutaneous process', questions the suitability of intradermal tests for contact sensitizers. It is noteworthy in the present context that he claims no more for his method than that it is an 'attractive test for detecting strong sensitizers among industrial chemicals'. It certainly is easy and rapid to perform.

An 8-year programme devoted to devising a more reliable method for detecting allergens culminated in the guinea-pig maximization test (GPM) devised by Magnusson and Kligman. This combines intradermal injection and topical application for induction, the choosing of a concentration of test agent based on its irritant effect and uses Freund's complete adjuvant as a means of increasing sensitization. The results achieved by this method have been compared with those obtained on parallel groups of guinea-pigs using the classical Landsteiner–Draize test. They have also been compared with the results obtained on human volunteers using the Kligman human maximization test (HMT) (220). Benzocaine, monobenzyl ether of hydroquinone, nickel sulphate and mercaptobenzo thiazole were among compounds which failed to sensitize by the Landsteiner–Draize test but were graded as potent sensitizers by the GPM test. All those substances tested which sensitized man, also sensitized the guinea-pig. With some substances the GPM test was more sensitive. For example, 20% of guinea-pigs were sensitized to *Vioform* compared with no humans, although this compound is known to be an undoubted, if only moderately potent, allergen. Known contact sensitizers which failed to react in the GPM test were lanolin and hexachlorophene.

Direct comparison of the tests is difficult because few compounds were

tested by all the workers and Voss in particular limited his observation to one class of chemicals. *Table VI* lists some of the substances tested by more than one investigator. It is clear that all the modifications are better than the original Landsteiner–Draize test at detecting not only weak or moderate but even potent human contact sensitizers. In so far as they can be compared, the guinea-pig maximization test would appear to achieve a higher degree of sensitization than Buehler's test but only a controlled comparison using several weak or moderate sensitizers would satisfactorily demonstrate their comparative efficiency. Similarly, Stevens' method cannot be fairly evaluated on the available evidence because few of the large number of compounds tested by him were also tested by the other investigators and he did not test compounds such as lanolin whose allergenic potential is of importance to the cosmetics industry.

In the GMT and Buehler's technique closed patches are applied to guinea-pigs either by enveloping the animal in a bandage unit or by keeping it in a specially designed restrainer. The first is not an easy operation and requires some skill in animal handling. In this respect there would be much to recommend Stevens' method if it is equally capable of detecting weaker allergens. There is, however, no doubt that the degree of sensitization achieved by the GMT is impressive and justifies the effort needed in using the test.

Human testing

Kligman (221) has made an admirable survey of standard methods of human tests for allergens. As he points out, a grave disadvantage of all such tests is that they cannot reproduce or replace ordinary usage of the product which is the only certain way of predicting the actual incidence of sensitization. Many months of use by genetically predisposed individuals may have to elapse before instances of allergic contact dermatitis are observed. The argument that laboratory tests should reproduce normal conditions of use is not entirely valid when trying to detect a weak or even moderate sensitizer. The test situation has to be exaggerated so as to attempt to reproduce the effect of normal (or even some abnormal) use over a prolonged period.

Standard methods including Kligman's are summarized in *Table VII*. Cutaneous application of the substance under test for a period of 24–48 h followed by a rest period and reapplication, repeated for 2 weeks form the basis of most techniques. After a rest period a challenge test is performed to ascertain if sensitization has occurred.

Table V. Guinea-pig tests for sensitizers

Method	Strain	Weight	No. of animals	Allergen		No. of injections	Site	Adjuvants
				Vol.	Concn.			
Landsteiner Jacob (201)	White	350–450 g	6–12 Male	0.1 ml	Varies 1/400–1/30 mg in 1% saline	Varied, e.g. 2/weekly 10 weeks	Into skin of back	No
Draize (214) (215)	Albino (Hartley)	300–500g	Usually 6–10 Male	0.1 ml	0.1%	Every other day or 3/week for total of 10	Intradermal. Back in area of 3–4 cm sq.	No
Voss (216)	Connaught or Hartley	300g	5–10 sex not specified	0·05 ml 0.1 ml	As high as possible	3/week to total of 10	Side (e.g. right) intradermal	No
Buehler (217)	Hartley (Hamm)	250 g	Male and female 10	0.5 ml	As high as possible in 2% ABS	6-h exposure one or more times according to nature of test material. 3/week for 3 weeks *or* 1/week for 6 weeks for weak sensitizers	Closed patch 7/8 × 1″. Applied to back and held by rubber dam (guinea-pig in restrainer).	No
Stevens (218)	Alderley Park Albino	2-months age	Housed individually 6–12	0.1 ml	0.25–10%	Daily for 3 days	Ear-flank. Topical painted on to skin	No (tried without effect)
Magnusson and Kligman 'Guinea-pig maximization test' (219)	Hartley	300–500 g	10–25	Stage 1 0.1 ml CFA 0.1 ml Test agent 0.1 ml Test agent + CFA		2 injections of each	Shoulder region. Area 4 × 6 cm 3 injections on each side	Yes
				Stage 2 7 days later 25% 2 × 4 cm filter paper covered substance in solution or in petrolatum		Closed patch for 48 h	Same area	

Table V. (continued)

Method	Rest period	Challenge			Comments
		Site	Dose	Scoring	
Landsteiner Jacob (201)	3 weeks	Flank l.d. skin	0.1 ml	Pink 'Elevated' etc.	Test substances included *p*-phenylenediamine Chloro and nitro benzenes *p*-nitroso methyl-aniline
Draize (214) (215)	2 weeks	Flank l.d.	0.05 ml 0.1 %	Reaction greater than average reaction following inducing injections	
Voss (216)	10–14 days	Opposite site (e.g. left) Intradermal Topical	0.05 ml (see comment) in Tween (see comments)	0–6 erythema to vesicles	Injection concentration and challenge concentration as high as could be used without causing excessive irritation or necrosis
Buehler (217)	2 weeks	Two sites 'local' and 'systemic'	Varies	0–4, 4= severe erythema	Concentration of test substance determined by its primary irritation threshold. Used at a concentration 'producing minimal amounts of erythema'. Same conc. used for challenge. ABS—aqueous tetrapropylene benzene sulphonate.
Stevens (218)	4 days (Challenge on 7th day of experiment)	1 cm diameter circles on flank	Range of concentrations 0·2 ml	24 h later Tr—tt	'Suitable vehicle' used for test substance. Most satisfactory: dimethyl-formamide. Moderately satisfactory: olive oil, paraffin oil and dinonyl phthalate.
Magnusson and Kligman 'Guinea-pig maximization test' (219)	2 weeks	Flank 5 × 5 cm area	Closed patch 24 h 25% in Petrolatum Liquids as is. See comments	24 h later 0–3 3 = intense redness and swelling	For induction and challenge substance always used at highest concentration which produces mild to moderate irritation. If non-irritating, area for topical applications (Stage 2) is pretreated with 10% sodium lauryl sulphate.

Table VI
Comparison of results obtained by various predictive animal tests

Experimenter	Number or % animals sensitized					
	Beuhler		Stevens	Magnusson–Kligman		
Method used	Landsteiner Draize	Buehler	Stevens	Landsteiner Draize	GMT	HMT
Compound or class of compound						
Tetrachlorsalicylanilide	0/10	8/10	—	2/25 (8%)	18/25 (72%)	88%
Allyl isothiocyanate	0/10	3/10				
α-Methylallyl isothiocyanate			0/8			
Phenyl isothiocyanate			4/8 (Trace reactions)			
Monobenzyl ether of hydroquinone	0/10	3/5		10/20 50%	10/20 50%	92%
p-Phenylenediamine	0/10	10/10	8/10			
Benzocaine	0/10	2/10		0/25 0%	7/25 28%	22%
Potassium chromate	1/10	1/10	0/8	3/20 15%	18/24 75%	100%
Formalin	1/10	3/10	5/8	1/20 5%	16/20 80%	72%
Nickel sulphate		0/10	—	0/20 0%	11/20 55%	48%
Nickel chloride			6/6			
Tween 80			Erythema reported in controls. Used as vehicle	0%	0%	0%

Table VII
Predictive human tests for contact sensitization

Test	No. of subjects	Allergen Amount/Conc.	Patch Size/Nature	Application No.	Application Duration	Application Rest period	Period before Challenge	Challenge	Comments
Schwartz-Peck (224)	200		¼″ sq. Occlusive	1	48 h		10d	Repeat standard patch test on new site. Read.	Old reliable and new formulae test at same time.
	Use test of 4 weeks with old and new formulae is part of complete test.								
Draize (215) (214)	200 Both sexes	0.5 ml or 0.5 g	1″ sq	10	24 h	24 h	10–14d	Repeat standard test on fresh site. Read.	Each patch placed on *fresh site.*
Shelanski Shelanski (225)	200	0.5 ml or 0.5 g	2.5 cm sq Occlusive *Same site*	10–15	24 h		14–21d	Repeat test on fresh site. Read	
Kligman (220)	25	1.0 ml liquid 25% by weight in petrolatum for nonirritant solids.	1.5″sq.	5	48 h	24 h		1″ square on back. Occlusive 0.4 ml of 10% SLS applied to skin for 1 h 24 h before application of test agent. 0.4 ml at 10% in petrolatum if this is non irritant.	Concentration of agent determined by its irritancy and is adjusted to the greatest concentration that will produce a moderate erythema in 48 h. For challenge the highest concentration producing *no* erythema.
				If substance is non-irritating patches of 5% sodium lauryl sulphate are applied during the 24-h interval or SLS is incorporated with the test agent.					

Kligman has shown that most earlier tests fail to predict the presence of weak or even moderate sensitizers, although the Shelanski test is an improvement on the original Draize technique. Kligman's modification is the only test which approaches results which give some realistic evaluation of the sensitizing capabilities of the test ingredient. In particular none of the earlier tests detected the sensitizing potential of Penicillin G, Streptomycin, Neomycin, Benzocaine, Furacin or Butyn Sulphate.

In the process of devising his test, Kligman (222) examined many factors influencing the induction of contact hypersensitivity. Among his more important conclusions are:

1. Inflammation enhances the degree of sensitization and for this purpose 5% sodium lauryl sulphate was the most effective agent either when applied alone before the test substance or incorporated with it.

2. Sensitization is more readily achieved by multiple applications to the same site. The test substance was applied to limbs for induction of hypersensitivity, but it is noted that the back is as 'sensitive as any other area' for challenge testing.

3. Topical application of the allergen is more effective than intradermal injection.

4. Sensitization is proportional to the amount of the allergen per unit area (surface concentration).

5. Sensitization rates are roughly proportional to the number of exposures especially with weaker allergens.

6. The optimal concentration for challenge is the highest which does not produce irritation up to a maximum of 10%.

7. Petrolatum is the best vehicle.

As an extension of his maximization induction method, Kligman (223) also recommends the use of a provocative patch test for improved detection of low degrees of sensitization. This consists in exposing the test site to the irritant effect of 10% sodium lauryl sulphate for 1 h, 24 h before applying the challenge patch test.

Even using the maximization test, Kligman obtained no instances of hypersensitization to lanolin, hexachlorophene or *p*-aminobenzoic acid. The status of *p*-aminobenzoic acid as an allergen has been disputed. Kligman's view is that it is an elicitor, i.e. not sensitizing in itself but producing positive reactions in individuals previously sensitized to some other members of the cross-reacting groups of chemicals. Kligman considers the allergenic potential of lanolin and hexachlorophene to be low and emphasizes that the maximization test is not sensitive enough to detect the 'feeble allergenic

potentiality of such substances'. The failure of a substance to produce sensitization by test does not 'signify absolute lack of allergenicity'. He does claim that the maximization test makes possible a grading of allergens and a realistic assessment of the risks attending their use.

Kligman had a panel of prisoners for testing. Undoubtedly, some of the stronger allergens produced unpleasant reactions and the exposure to these and the somewhat irksome testing regimen might be not well tolerated by the average panel of volunteers as used by industry.

In vitro *tests*

The possibility of testing the allergenic potential of compounds by *in vitro* tests has obvious attractions but to date no reliable method has been evolved. The three techniques that have been reported rely on the changes occurring in allergized cells when in contact with the responsible allergen. It should be noted that this presupposes a sensitized animal or human as a source of such cells. The respective techniques are:

Lymphocyte transformation test (182, 183)

Sensitized peripheral human blood lymphocytes will undergo blast transformation if cultured with the appropriate allergen even in low concentration.

Circulating lymphocytes of guinea-pigs sensitized to dinitrochlorbenzene also undergo this transformation when in contact with DNCB (36). Baumgarten and Geczy (240) have also induced delayed hypersensitivity by injecting intraperitoneally autologous, dinitrophenylated lymphocytes. Union with lymphocyte protein may well be an important or essential property of a hapten capable of inducing delayed type hypersensitivity. In the quoted experiment sensitization was not achieved with dinitrophenylated erythrocytes, serum proteins or killed lymphocytes.

Lymphocyte cytotoxicity (185)

Delescluse and Turk found peripheral lymphocytes from guinea-pigs with contact sensitivity to DNFB were cytotoxic to chicken red cells conjugated with DNFB and then labelled with ^{51}Cr. Cytotoxicity was determined by release of ^{51}Cr from the red cells.

Inhibition of macrophage migration (40, 184)

David (40) has developed a technique for detecting delayed sensitivity in

the guinea-pig by the inhibition by the allergen of macrophage migration from capillary tubes. This again depends on the presence of allergized lymphocytes.

Most of these types of *in vitro* techniques have been studied using cell samples from animals or humans sensitized either to tuberculin or to potent chemical sensitizers like DNCB or DNFB. Their future usefulness will depend on their reproducibility with moderate and weak allergens and their ability to detect these with more efficiency than the standard tests. This is an obvious potential field for future research.

'In use' tests

Schwartz and Peck stressed the necessity of an 'in use' test to complement their patch testing. Usually 200 volunteers are used This number has been shown repeatedly to be of very little statistical value when the test agent is a very weak allergen only likely to produce sensitivity in 1 in 1000 users. It would be of interest to know how often these 'in use' tests provide data on sensitization not provided by animal testing and human tests of the repeated insult variety.

Tests for types I and III sensitivity

Attempting to deliberately produce Type I sensitivity in human volunteers would be completely unethical because of the hazards involved.

As already noted, an exact animal model for human Type I hypersensitization is not readily available. Patterson (25) has reviewed the possibilities exhaustively.

Factors which make an experimental reproduction of this type of hypersensitivity particularly difficult are:

1. The rarity of 'atopic' experimental animals. Although guinea-pig (226, 227), rat (228), rabbit (229–231) and mouse (232, 233) under experimental conditions produce anaphylactic antibodies with electrophoretic mobilities different from those of their non-anaphylactic antibodies the former have not been shown to arise naturally except, as already mentioned, in response to infection with intestinal parasites. Atopic dogs do approximate more closely to the human atopic individuals, and Rockey and Swartzman (234) have succeeded in inducing an antidinitrophenyl reaginic type antibody in these animals.

2. Injection (as opposed to inhalation or ingestion) into an atopic human, or presumably animal, may result in the development of a heat

stable, IgG type antibody with no Type I affinity for skin and which, therefore, will fail to induce passive cutaneous anaphylaxis in homologous animals. This type of antibody (sometimes known as blocking antibody) is produced in hay fever patients during desensitization. Such *treated* patients have naturally produced IgE antibody and induced IgG antibody to the same allergen. A similar situation exists when, for example, guinea-pigs are injected experimentally with a potential Type I-producing antigen. However, they produce two (if not more) antibodies, one heat labile and anaphylactic and the other heat stable, non-anaphylactic and complement fixing.

3. IgG antibodies of the heat stable, non-anaphylactic type with no affinity for skin produced in animals *other* than the guinea-pig may produce passive cutaneous anaphylaxis in this animal which seems uniquely susceptible in this respect. Heat labile, anaphylactic type antibodies only induce passive cutaneous anaphylaxis in animals of the same or closely allied species.

4. Landsteiner and Chase (235) in their early experiments consider that the ability to unite with *serum protein* is an important factor in determining whether an injected hapten will induce anaphylactic antibodies. Obviously a given hapten might react with the proteins of some species and fail to react with others, so any experimental findings cannot be automatically applied to all other species (including man).

Despite all the above difficulties, any protein or hapten found to produce Type I hypersensitivity experimentally in the guinea-pig is liable to produce Type I hypersensitivity in atopic individuals, especially if inhaled or ingested. The same allergens may theoretically be implicated in Type III hypersensitivity in non-atopic (or atopic) individuals.

Hartley guinea-pigs are particularly susceptible to acute anaphylaxis (236). They can be immunized by various techniques. A single subcutaneous injection of 0.01–1 mgm of protein may be successful. More satisfactory are a series of three to five injections at 4- to 7-day intervals. Landsteiner and Chase (235) injected picryl chloride daily for 15 days, but unless Freund's adjuvants are used the quantities of antibodies, especially to haptens or hapten conjugates are usually low.

Type I hypersensitivity in the guinea-pig can be demonstrated by:

Local challenge of sensitized animals

Intradermal injection of allergen plus intravenous injection of Trypan blue. A positive reaction is an accumulation of dye at the site of injection.

Passive cutaneous anaphylaxis

Serum from a sensitized guinea-pig is injected intracutaneously into a non-sensitized animal. The allergen and trypan blue are given intravenously. As above, a positive reaction is an accumulation of dye at the site of serum injection within 15 min.

Pulmonary challenge

Very small amounts of the specific allergen administered as an aerosol in a chamber will give rise to coughing and respiratory distress.

Isolated uterine muscle or intestinal strips

Isolated uterine muscle or intestinal strips from an actively or passively sensitized guinea-pig will contract if immersed in a saline bath containing the allergen.

EVALUATION OF TEST RESULTS

Throughout it has been assumed that it is desirable to know if a compound to be used in a cosmetic product is a sensitizer, irrespective of its concentration in the final product or the way in which that product is designed to be used. This is deliberate. Unlike irritants, sensitizers can evoke reactions in sensitized persons even when present in very low concentrations. This concentration may be such that the chances of the product producing sensitization *de novo* on its own is minimal, but if the same ingredient is one likely to be encountered in other, possibly quite different products, sensitization may well have been induced by the use of these and the cosmetic be wrongly blamed as the primary cause of the contact dermatitis. An increasing number of hypersensitized individuals exist in the population and an increasing number are aware of the individual allergens to which they react. A warning that a cosmetic contains a potential allergen such as, for example, lanolin, would avoid much discomfort to the customer and possible complaints to the manufacturer.

The decision whether or not to use an allergen in a product can be a difficult one. Obviously no strong or moderately strong allergen should be included unless it is absolutely essential to the production of the desired effect, in which case adequate warning should be, and in the instance of some chemicals such as paraphenylene diamine, legally must be given. Frequently, however, it is a question of a product desirable to 99.99% of the population being of potential hazard to less than 0.01% because it contains a

known or suspected weak allergen. To exclude completely the use of such substances as lanolin or parabens would be unjustified, but nevertheless, knowledge of the risks involved is desirable. In such instances testing a product under its normal conditions of use and with the particular ingredients at their normal concentration would almost certainly fail to reveal its sensitizing capability.

Some allergens, such as eosin, lanolin and neomycin may not penetrate the stratum corneum in sufficient amounts to produce a reaction (237) unless tested under the exaggerated conditions of a maximization or similarly stringent test. Although, as we have seen, even these tests may be unsuccessful they do at least increase the chances of detecting weak allergens and enable a more realistic assessment of the situation to be taken.

Conclusions

Immunologists, dermatologists and cosmetic manufacturers inevitably view the induction of hypersensitization by chemical substances in a very different light. What is a fascinating theoretical problem to the immunologist, a very practical clinical matter to dermatologists concerned with their patients' welfare may be a very irritating problem for a cosmetic manufacturer with no compensating professional or commercial interest. All he can hope is that a conscientious approach to the problem will earn a good reputation for his wares and an increase in custom. There is no magic formula whereby a potential allergen can be detected easily and with certainty. Animal tests if competently performed will screen out the stronger allergens and provide a basis for further human tests. These again will provide a further check that no potent allergen is present in the product. If a stringent test such as recommended by Kligman is used, all but weak allergens will be detected. No method exists which will reliably detect these. In a joint study of 4000 patients with dermatoses in eight European dermatological clinics, 2.6% of patients had positive patch tests to wool alcohols—derivatives of lanolin (238, 239). Lanolin would have passed all the screening methods outlined here. Of the same patients 1.9% reacted positively to parabens and 4.9% to *p*-phenylene diamine. The first were for many years not regarded as allergens at all; the latter is one of the few cosmetic ingredients classifiable on all tests employed as a strong allergen.

This highlights the problem. A strong allergen used in a few specialized products may be a much smaller risk than a weak sensitizer in a wide range of products used daily by many customers. In the above survey, undoubtedly,

cosmetics alone did not account for the high percentage of positive reactions to parabens and lanolin which are also present in medicaments.

The only safeguard is a good testing programme before a new product is launched, followed by an adequate test market survey and a conscientious follow up of all relevant consumer complaints that may come at this early stage or later after a product has been on the market for some time. A thorough literature search may reveal that a new proposed ingredient is closely allied chemically to known allergens. This should immediately engender caution.

Finally, there is hope that *in vitro* methods may be perfected in the future that will prove to be both simple and reliable methods of detecting all grades of allergens.

(Received: 25th August 1972)

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