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THE IDENTIFICATION OF CONTACT ALLERGENS BY ANIMAL ASSAY. THE GUINEA PIG MAXIMIZATION TEST*

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The guinea pig is the laboratory animal *par excellence* for the establishment of allergic contact sensitization. Basic knowledge of this form of delayed hypersensitivity has derived almost exclusively from studies of this animal using potent allergens such as dinitrochlorobenzene and p-nitroso-dimethylaniline.

It is the consensus however that the guinea pig is less sensitizable than the human. The key difficulty is that some, perhaps many, substances which are known to be troublesome human sensitizers have failed to sensitize the guinea pig. These are generally weaker allergens such as penicillin, neomycin, and heavy metals. This deficiency seriously compromises the usefulness of this animal in screening new substances for their allergenic potentialities. On the other hand, there seem to be no instances in which a substance sensitizing the guinea pig fails to do so in the human. It is obviously desirable to use animals rather than humans in preliminary screening but until false negative results can be eliminated, guinea pig testing cannot be relied upon to identify contact allergens. Investigators have been much concerned with closing the sensitivity gap between the guinea pig and the human.

The Draize test (1, 2) recommended by the U.S. Food & Drug Administration, is perhaps the most widely used, especially by industries producing new chemicals. Its fundamental design is that of Landsteiner & Jacobs (3) in their renowned study of experimental contact dermatitis. A series of ten intradermal injections is given on alternate days and the animals challenged intracutaneously two weeks after the last injection. The literature does not contain data which would enable one to appraise accurately either its strengths or weaknesses, except for spoken judgements of too frequent failures.

Divers efforts have been made to elevate the sensitivity of the guinea pig. Chase (4) established an exquisite degree of sensitivity to picryl chloride by his "combination" method. First picrylated erythrocyte stromata in adjuvant was injected, followed by a series of weekly contact tests with picryl chloride in which each exposure progressively intensified the degree of sensitivity. This procedure is suitable for chemicals which can couple with red blood cells; it is probably too cumbersome for routine screening.

Voss (5) improved the Draize test by using the highest concentration of the test agent that was non-irritating instead of a fixed concentration of 0.1%. Even so, of 44 mercaptans studied, eleven sensitized men but failed in guinea pigs.

Still better results were secured by Buehler (6) who compared the Landsteiner intradermal test to repeated topical applications by closed patch. The animals were restrained for 6-hour periods during the topical exposures. The superiority of occlusive topical exposure was very evident with a number of substances which did not sensitize by injection: *viz.*, tetrachlorosalicylanilide, monobenzyl ether of hydroquinone, benzocaine, thioglycerol, and others. However, sensitization to salts of mercury, cobalt and nickel was not obtained though these are well-known allergens in humans.

Chase and Maguire (7) have elaborated the "combination" method into a "split adjuvant" technique. Typically 5 sites are injected intradermally with paraffin oil containing killed tubercle bacilli. Each site is reinjected with the allergen 24 hours later. A subsequent series of patch tests boosts the sensitivity to a very high level. Although these workers demonstrated the possibility of inducing an exquisite state of sensitization to dinitrochlorobenzene, picric acid, and picryl chloride, the prospect they hold forth of using the split adjuvant technique to identify contact allergens would seem to be somewhat spoiled by their allusion

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to indifferent results obtained with weaker sensitizers such as formaldehyde, penicillin, and iodoform. However, the specifications of this technique are not yet fixed and further modifications may well demonstrate an improved capacity to detect weak allergens. Hood's method (8) has apparently proved satisfactory in an industrial setting (du Pont). The design calls for thrice weekly topical applications for 9 exposures using the highest concentrations which do not excessively irritate. The applications are made to abraded skin in half the test group while all animals receive a total of 4 intradermal injections over a 3-week period.

We mounted an extensive research which had the prime objective of enhancing the usefulness of the guinea pig in screening contact allergens. The evolution of this work over an 8-year period has followed the tactics and principles utilized in developing the maximization test for identifying contact allergens in humans (9, 10, 11, 12). The variables which control the induction and elicitation of contact sensitization have been studied quantitatively; these results will be the subject of a forthcoming monograph. The knowledge gained has been combined into a test procedure which we now consider highly reliable for detecting allergenic chemicals.

The purposes of this paper are: (1) to provide specifications for the performance of the guinea pig "maximization test", (2) to compare the sensitivity of this procedure to that of the Landsteiner-Draize test and (3) to correlate the results of human and guinea pig maximization testing.

MATERIALS AND METHODS

Animals. Albino guinea pigs weighing 300–500 grams are used. It is important to avoid older animals since they are appreciably less sensitizable. While susceptibility is not influenced by sex, we prefer females because of their greater tractability. The combativeness of males often damages the test sites. Pregnant animals are entirely unsuitable because of decreased capacity to manifest an inflammatory reaction.

The standard outbred Hartley strain should be used unless the investigator has empirically verified the equivalent sensitizability of another genotype. Although Chase (13, 14) and recently Polák *et al.* (15) have clearly demonstrated the possibility of selecting genotypes with either increased or decreased susceptibilities to specific allergens, most breeds should be acceptable because the antigenic dose is extreme.

Though most of our basic studies have been conducted on groups of 25 animals, it seems likely that ten will generally suffice for preliminary screening. If none become sensitized or, conversely, nearly all become allergic, one may confidently certify the chemical to be a weak at best or strong allergen, respectively. A result between these extremes may justify expanding the sample to secure more accurate appraisal.

Test substances. The agents included substances not known to sensitize humans: aluminum chloride, sodium lauryl sulfate and Tween 80, as well as those which could be clinically rated as strong sensitizers, *e. g.* formalin and streptomycin. Most of the test agents have mild to moderate allergenicity in man.

LANDSTEINER-DRAIZE (L-D) TEST

The same battery of allergens was tested by the L-D method in order to compare the efficiency of the two procedures. The procedure was as follows: A 0.1% solution or suspension of the test material in saline was injected intradermally into male albino guinea pigs of 300–500 grams. Injections of 0.1 ml were made every other day or three times a week for a total of ten, keeping the injections within a field 3 to 4 cms square. The site was read 24 hours after each injection. Two weeks after the 10th injection, the animals were challenged by an intradermal injection of 0.05 ml into a fresh skin area. The animal was judged to be sensitized if the reaction was clearly greater than the average reaction of the inducing injections.

SPECIFICATIONS OF THE GUINEA PIG (G.P.) MAXIMIZATION TEST

Preparation of Test Material for Induction

A. Intradermal injections. Injections are made with the allergen incorporated in Freund's adjuvant and also independently. It is simplest to purchase Freund's Complete Adjuvant; we have found that the Difco product[1] gives results entirely comparable to the emulsion prepared according to Freund's original description (16).

Immediately before injection the emulsion is prepared by blending the commercial adjuvant with an equal volume of water. The adjuvant is placed in a container and the aqueous phase is added in several installments while homogenizing with a rotating stirrer. Water soluble

[1] Difco Laboratories, Detroit, Mich.

allergens are first dissolved in the water phase; oil soluble or insoluble chemicals are dissolved or suspended in the adjuvant (a mixture of paraffin oil and an emulsifier with mycobacteria). The final concentration of the allergen is 5% by weight *provided* that the injection does not produce local necrosis or ulceration and is sufficiently free of systemic toxicity as to not impair the health of the animal. Otherwise, the concentration is adjusted to the highest level that can be well tolerated locally and generally; this will usually fall within the 1–5% range.

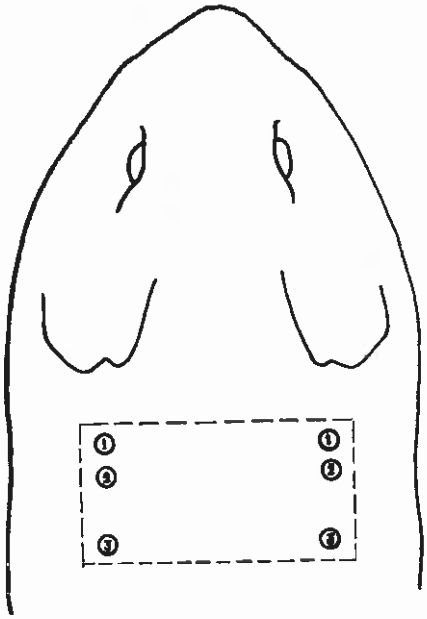

FIG. 1. *Induction.* First stage. A row of three injections are made on each side: (1) 0.1 ml of adjuvant alone, (2) 0.1 ml of test substance alone and (3) 0.1 ml of the test agent emulsified in the adjuvant. The rectangle outlines the area to which the test substance will be applied topically one week later.

The allergen which is to be injected without adjuvant is dissolved or suspended in an appropriate vehicle, water if it is soluble in that medium. Insoluble substances are incorporated into either paraffin oil, peanut oil or propylene glycol, whichever enables the best solution or dispersion.

B. Topical application. Solids are finely pulverized and incorporated in petrolatum at 25% concentration by weight if not excessively irritating or deleterious to general health. Otherwise the concentration is the highest one which produces a mild to moderate irritation.

Liquids are used at the highest concentration which does not produce excessive inflammation, undiluted if not irritating. Otherwise the concentration in petrolatum or water should be so adjusted as to produce a mild to moderate irritation.

Induction Procedure

Induction is a two-stage operation. First, 3 pairs of injections are made simultaneously. See Figs. 1 and 2. Second, closed patch exposure is performed over the injection sites one week later. See
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[3] Model X 555 M, Su
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[4] W. & R. Balston Ltd.
[4] Minnesota Mining &
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[5] T. J. Smith & Neph
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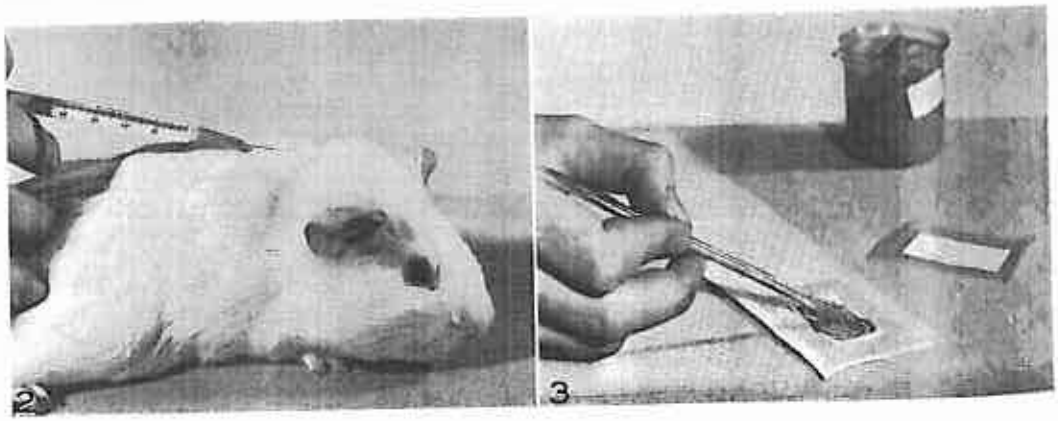

FIG. 2. *Induction.* First stage. An area of 4 × 6 cm over the shoulders is clipped short with an electric clipper. Into this area three pairs of symmetrical intradermal injections are given simultaneously as diagrammed in fig. 1.

FIG. 3. *Induction.* Second stage Preparation of the patch. A 2 × 4 cm filter paper patch is loaded with the test substance, backed successively by the impermeable plastic tape and the elastic bandage.

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one week later. See Figs. 3, 4 and 5. The shoulder region is the induction site. An area 4 × 6 cm is clipped short with an electric clipper.

A. Intradermal injections. A row of 3 injections, six in all, are made on each side as follows: (1) 0.1 ml of the adjuvant without the test agent, (2) 0.1 ml of test agent without adjuvant and (3) 0.1 ml of the test substance emulsified in complete adjuvant (Fig. 1). It should be noted that the injection sites are just within the boundaries of the 2 × 4 cm patch, which will be applied one week later.

The adjuvant injections should be made deep into the dermis to minimize sloughing.

B. Topical application. One week after the injections the same area is clipped and shaved closely with an electric razor. We find the Sunbeam Shavemaster[2] most suitable for the latter operation. If the test agent is non-irritating, the area is pretreated with 10% sodium lauryl sulfate (SLS) in petrolatum 24 hours before the patch is applied. The SLS is massaged into the skin with a glass rod without bandaging. This concentration of SLS enhances sensitization by provoking a mild inflammatory reaction.

The test agent in petrolatum is spread over a 2 × 4 cm patch of Whatman No. 3MM filter paper[3] in a thick even layer or, if liquid, to saturation. The patch is covered by an overlapping impermeable, plastic adhesive tape (1½" 3M Blenderm[4]). This in turn is firmly secured by elastic adhesive bandage (Tensoplast[5], 6.4 cm in width), wound around the torso of the animal. This dressing is left in place for 48 hours (Figs. 3, 4 and 5).

It is expedient to prepare beforehand all the occlusive bandage units required for one session. Lengths of elastic bandage, about 25 cm long, are cut and placed with the adhesive surface up on the worktable. A 6 cm strip of the impermeable plastic tape, adhesive side up, is applied to one end of the elastic bandage. Finally, the patch is placed centrally on the plastic tape and loaded with the test substance. With fluids, however, it is best to place the wetted patch directly on the skin and then apply the Blenderm-Tensoplast covering.

[2] Model X 555 M, Sunbeam Electric Ltd., Nerstone, East Kilbridge, Glasgow, Scotland.
[3] W. & R. Balston Ltd., Maidstone, England.
[4] Minnesota Mining & Manufacturing Co., St. Paul, Minn.
[5] T. J. Smith & Nephew Ltd., Hull & Welwyn Garden City, England.

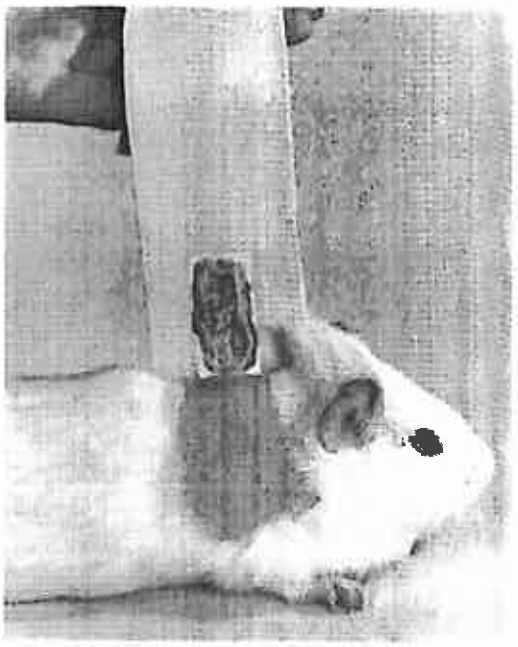

FIG. 4. *Induction.* The occlusive bandage unit with the loaded patch is applied over the sites injected a week earlier.

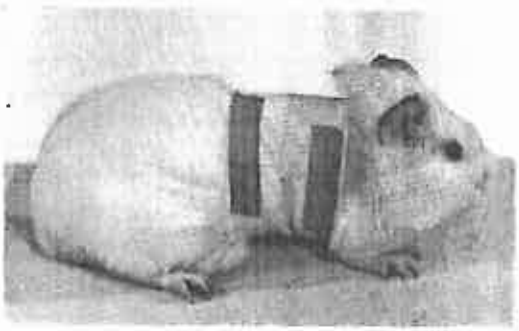

FIG. 5. *Induction.* The occluded patch is firmly secured by elastic adhesive bandage, wound around the shoulder region of the animal and left in place for 48 hours.

This study was conducted under the sponsorship of the Swedish Medical Research Council (Project No. 19X-1036-01) and was supported, in part, by funds from "Edvard Welanders stiftelse", and "Riksförbundet mot allergi" (to B. Magnusson).

Challenge Procedure

Challenge is by topical application. Provided there is no irritation, solids are incorporated in

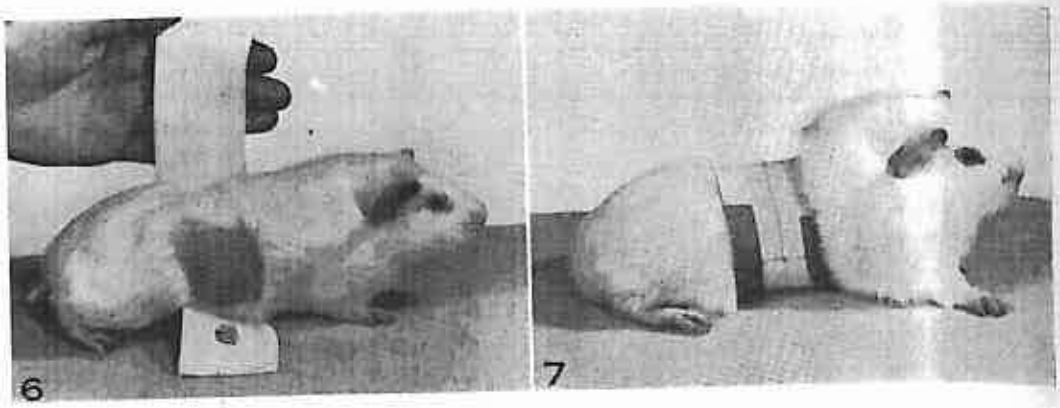

FIG. 6. *Challenge*. The challenge test is performed on a 5 × 5 cm clipped and shaved area of the flank. The test agent is applied on a 2 × 2 cm piece of filter paper under a sealed dressing as for induction.

FIG. 7. *Challenge*. The occluded patch is firmly secured by an encircling elastic adhesive bandage for 24 hours.

petrolatum at 25% concentration and liquids are used as is. Otherwise a sub-irritating concentration is empirically found which will not cause redness in any of ten unexposed animals. It is essential to avoid toxic concentrations in order to eliminate false positive readings.

The animals are challenged two weeks after the topical induction. Hair is removed from a 5 × 5 cm area on the flank by clipping and shaving as before.

The test agent is applied on a 2 × 2 cm piece of filter paper in the same fashion as for topical induction. The patch is sealed to the flank for 24 hours under a 4 cm strip of 1½" Blenderm (Fig. 6). This in turn is secured by Tensoplast wound around the trunk (Fig. 7). The importance of a secure dressing which affords complete occlusion cannot be too strongly emphasized.

Reading of challenge reactions. The challenge site is evaluated 24 hours after removal of the patch. Any irritation produced by the plastic tape will usually have subsided by then and the allergic reaction will generally be at its peak. The sites are again examined in an additional 24 hours, mainly to detect weak, slowly developing reactions.

Three hours prior to the first reading, the test site is shaved with the electric razor and the skin gently cleansed of excess chemical with ether. The readings are preferably made in indoor daylight at noon. Artificial light sources[a] are obtainable which simulate "daylight".

Redness constitutes the minimum criterion of an allergic reaction. This presupposes of course that identical tests on non-sensitized animals cause no reaction. Uncertainty concerning the validity of mild reactions may be reduced by rechallenging within three to four days. Histologic examination can usually distinguish between allergic and irritant responses if doubt still persists (17, 18). Strongly sensitized animals display a vivid redness, associated with indurated swelling. If desired one can score the reactions on a 4-point scale: no reaction, 0; scattered mild redness, 1; moderate and diffuse redness, 2; intense redness and swelling, 3. The important statistic in maximization testing however, is the frequency of sensitization not intensity.

Rating of allergenicity. Based upon the percentage of animals sensitized we assigned each substance to one of five grades of allergenic potency ranging from 0 to weak (I) to extreme (V) (Table I). We could thus judge whether the results of maximization testing were similar in humans and guinea pigs.

RESULTS

Twenty-four substances of differing allergenicity were assayed by both procedures con-

[a] Manufactured by Macbeth Corporation, P.O. Box 950, Newburgh, New York.

TABL

Maximizatio

Sensitization rate (%)	Grade
0–8	I
9–28	II
29–64	III
65–80	IV
81–100	V

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Eleven known allergens
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Substance
Acrylic monomer
Aluminum chloride
Apresoline®
Atabrine®
Benzocaine§
Formalin
Hexachlorophene
Lanolin
Malathion®
Marfanil®
Mercaptobenzothiazole
Mercuric chloride
Monobenzyl ether of hyd
Neomycin
Nickel sulfate
Penicillin G
Potassium dichromate
Sodium lauryl sulfate
Streptomycin
Sulfathiazole
Tetrachlorosalicylanilide
Turpentine
Tween 80
Vioform®

* vehicle = H_2O.
† vehicle = ethanol 70

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TABLE I

Maximization grading

Sensitization rate (%)	Grade	Classification
0–8	I	Weak
9–28	II	Mild
29–64	III	Moderate
65–80	IV	Strong
81–100	V	Extreme

comitantly. The results are given in Table II. There was a startling disparity between the capabilities for identifying contact allergens. Eleven known allergens (Benzocaine®, Malathion®, mercaptobenzothiazole, mercuric chloride, monobenzyl ether of hydroquinone, neomycin, nickel sulfate, streptomycin, sulfathiazole, turpentine, and Vioform® failed to sensitize a single animal by the L.-D. test. Maximization testing readily identified these, a majority of animals usually becoming sensitized. Neither technique was successful with lanolin and hexachlorophene, marginal sensitizers at best. The maximization procedure unequivocally identified every clinically significant allergen. No reactions were obtained with non-allergens, *viz.* sodium lauryl sulfate, Tween 80 and aluminum chloride.

Table III compares the allergenicity grades achieved by the L-D test with those of maximization testing in guinea pigs and humans.

TABLE II

Comparison of Landsteiner-Draize with maximization test

Substance	Maximization Test				Landsteiner-Draize Test
	Induction		Challenge	Sensitization Rate	Sensitization Rate
	Intradermal Concentration in Adjuvant	Topical Concentration in Petrolatum	Topical Concentration in Petrolatum		
			%		
Acrylic monomer	5	5	10*	21/25	1/25
Aluminum chloride	2	25	2	0/25	0/25
Apresoline®	2	5	1	16/20	6/20
Atabrine®	1	25	10	18/20	5/20
Benzocaine®	2	25	5	7/25	0/25
Formalin	5	5*	2*	16/20	1/20
Hexachlorophene	5	25	1.5	0/25	0/25
Lanolin	5	25	15	0/25	0/25
Malathion®	10	10	20	13/24	0/20
Marfanil®	5	5	20	20/20	6/20
Mercaptobenzothiazole	1	25	15	8/20	0/20
Mercuric chloride	0.1	1	0.1*	8/25	0/25
Monobenzyl ether of hydroquinone	0.5	25	25	10/20	0/20
Neomycin	25	25*	25*	18/25	0/25
Nickel sulfate	5	5*	0.5*	11/20	0/20
Penicillin G	3	5	10	20/20	7/20
Potassium dichromate	1	1	0.1*	18/24	3/20
Sodium lauryl sulfate	1	5	0.5	0/25	0/25
Streptomycin	10	10	0.5*	18/25	0/25
Sulfathiazole	5	25	10	0/25	0/25
Tetrachlorosalicylanilide	5	1	1†	18/25	2/25
Turpentine	5	25	20	16/25	0/20
Tween 80	5	25	20	0/25	0/25
Vioform®	5	25	5	5/25	0/25

* vehicle = H_2O.
† vehicle = ethanol 70%.

TABLE III

Grades of allergenic potency by the Landsteiner-Draize test, the maximization test in humans and the maximization test in guinea pigs

Substance	Landsteiner-Draize Test		Guinea Pig Maximization Test		Human Maximization Test[1]	
	% pos.	Grade	% pos.	Grade	% pos.	Grade
Acrylic monomer	4	I	84	V	ND	
Aluminum chloride	0	I	0	I	0	I
Apresoline®	30	III	80	IV	100	V
Atabrine®	25	II	90	V	78	IV
Benzocaine®	0	I	28	II	22	II
Formalin	5	I	80	IV	72	IV
Hexachlorophene	0	I	0	I	0	I
Lanolin	0	I	0	I	0	I
Malathion®	0	I	54	III	100	V
Marfanil®	30	III	100	V	ND	
Mercaptobenzothiazole	0	I	40	III	38	III
Mercuric chloride	0	I	32	III	92	V
Monobenzyl ether of hydroquinone	0	I	50	III	92	V
Neomycin	0	I	72	IV	28	II
Nickel sulfate	0	I	55	III	48	III
Penicillin G	35	III	100	V	67	IV
Potassium dichromate	15	II	75	IV	100	V
Sodium lauryl sulfate	0	I	0	I	0	I
Streptomycin	0	I	72	IV	80	IV
Sulfathiazole	0	I	36	III	4	I
Tetrachlorosalicylanilide	8	I	72	IV	88	V
Turpentine	0	I	64	III	72	IV
Tween 80	0	I	0	I	0	I
Vioform®	0	I	20	II	0	I

[1] Results from Kligman (1966d).
ND = not done.

The L-D test rated 14 substances as weak allergens (Grade I) whereas 12 of these had grades of II or more by maximization testing. Moreover, no substance was graded higher than III by the L-D test whereas fully 10 achieved that status by the maximization test.

As regards maximization testing in guinea pigs and humans, the results are remarkably congruent (human data from Kligman (12)).

Agents which sensitized humans invariably did so in the guinea pig. The quantitative similarities are noteworthy. The ratings for the two tests were within a single grade level for 18 of the test substances; for the other four the discrepancy was two grades. Vioform® sensitized guinea pigs but not man.

DISCUSSION

The guinea pig maximization procedure apparently detects and rates allergenic substances in a way comparable to that of the human maximization assay. The procedure has proved both specific and sensitive. In regard to sulfathiazole and Vioform® the G.P. test was even more sensitive. The latter was entirely missed in humans but achieved grade II status in guinea pigs. Grade I for sulfathiazole on human testing doubtless underrates its allergenic potentiality; grade III in guinea pigs seems more in accord with clinical experience.

In a total experience which is larger than the results presented here, specificity of the test has been upheld. Guinea pigs do not become sensitized to substances which do not induce contact allergy in humans.

Although we are persuaded that the guinea pig test can identify contact allergens as reliably as the human, it is all too easy to make misjudgements. If unwarranted conclusions are to be avoided one must clearly understand what

st, the maximization test in humans ea pigs

	Guinea Pig Maximization Test		Human Maximization Test[1]	
	% pos.	Grade	% pos.	Grade
	84	V	ND	
	0	I	0	I
	80	IV	100	V
	90	V	78	IV
	28	II	22	II
	80	IV	72	IV
	0	I	0	I
	0	I	0	I
	54	III	100	V
	100	V	ND	
	40	III	38	III
	32	III	92	V
	50	III	92	V
	72	IV	28	II
	55	III	48	III
	100	V	67	IV
	75	IV	100	V
	0	I	0	I
	72	IV	80	IV
	36	III	4	I
	72	IV	88	V
	64	III	72	IV
	0	I	0	I
	20	II	0	I

y comparable to that of the human ation assay. The procedure has proved ecific and sensitive. In regard to sul- e and Vioform® the G.P. test was even nsitive. The latter was entirely missed ns but achieved grade II status in igs. Grade I for sulfathiazole on human doubtless underrates its allergenic po- ; grade III in guinea pigs seems more l with clinical experience.

total experience which is larger than ılts presented here, specificity of the been upheld. Guinea pigs do not be- ensitized to substances which do not ontact allergy in humans.

ıgh we are persuaded that the guinea can identify contact allergens as reli- the human, it is all too easy to make ements. If unwarranted conclusions are oided one must clearly understand what

kind of decisions are permissible. Sound interpretation requires both judgement and experience. Our views have been presented previously (12).

The aim of the test clearly defines its limitations. It simply establishes to what extent a particular substance has the *potentiality* for acting as a contact sensitizer. It reveals that a chemical possesses immunogenic capabilities but the percentage of animals sensitized does not indicate the probable human incidence of sensitization. The antigenic stimulus in the test procedure is enormously greater than under conceivable conditions of use; this magnification is necessary in order not to miss weak allergens. Whereas the L-D test seriously underestimates the hazard by failing to identify fairly potent sensitizers, the G.P. maximization test may mislead the unforewarned into an overestimation of risk.

Actually there is one particular result which is predictive and enables a rather firm estimate of safety in use; this is when none of the animals becomes sensitized. This indicates an allergenic potential so low that no imaginable human exposure is likely to be attended by a significant incidence of sensitization. We emphasize that it does not mean that the substance will never sensitize anyone but rather that the probability of sensitization is very low.

Interpretation becomes more troublesome when a high porportion of the animals becomes allergic. Let it be stated forthwith that this outcome does not necessarily compel one to abandon interest in the substance. This result merely warns the toxicologist of the possibility of harmfulness. Whether the agent should be discarded or studied further requires careful consideration of many factors. These include whether the material is to be used in high or low concentration, whether for a short or long period, whether it will be applied to normal or diseased skin, whether it is likely to be leached out of the product, whether its effects are so unique and advantageous that even an appreciable risk is justified.

If a substance is found to be a potent allergen but has virtues which merit continuing interest, we would propose the following guidelines in estimating the hazard. Instead of the chemical itself, it can be tested in the form and concentration in which it will be actually used, viz. as a cosmetic, a topical drug, a fabric finisher, an insecticide, etc. The end product, not the chemical itself, is assayed. If this results in little or no sensitization, exaggerated exposure testing in humans would be a likely next step. One might apply the product five times daily instead of once, or perhaps under occlusion or in overly generous amounts to large areas, or perhaps to skin deliberately damaged by a chemical irritant. So varied are the applications of substances to human skin that one cannot lay down the conditions of further testing in anything more than general terms.

Such exaggerated use or stress testing provides a safety factor in deciding to go ahead with commercial exploitation even if one or more ingredients are known to be potent allergens.

Finally, the timid should be apprised that certain substances known to be moderate to strong sensitizers by maximization testing are in fact in widespread use. Examples of these are neomycin, penicillin, streptomycin, Malathion®, and p-phenylenediamine.

Res ipsa loquitur!

SUMMARY

A new procedure has been described, the guinea pig maximization test, for identifying contact sensitizers. Injections are given intradermally with and without complete Freund's adjuvant and one week later the test agent is applied topically over the injection site. The animals are challenged by patch test two weeks later.

The sensitizing potentialities of about twenty allergens of differing potencies were determined concomitantly by the maximization and Landsteiner-Draize procedures. The sensitivity of the latter was quite low, eleven substances failed to sensitize a single animal although these were clearly allergenic by the maximization test.

The results of maximization testing in the guinea pig were quite comparable to humans. Human allergens invariably sensitized the guinea pig.

Guidelines are set forth for interpreting the results and obtaining further data to estimate the hazard of clinical sensitization in use.

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THE EFFEC SEVERI

RONALD F. HAC

Local protection of the skir damage, by topical chemical a considerable interest from both cal and radiotherapeutical pc number of substances, e.g. me (MEA), have been found wh skin radioprotectors when adn travenous or subcutaneous inj erence 1 for review). Attempts tion skin damage by topical tre been very successful. Unfortu nous injection affords systemic subcutaneous infiltration is t also offer some generalized prot

Several properties of din (DMSO) suggested its possibl topical skin radioprotector. F shown to be a radioprotector animal (2) and cellular (3) I passes through intact skin w (4). Third, it can act as a pe trant carrier for a number of (5). For this latter reason, in DMSO alone, experiments were DMSO plus MEA (a very effe protector), and with DMSO (in an attempt to decrease loc and hence afford protection). established however, that DM penetrant carrier for either M rine.

MATERIALS AND ME

Male rats (Simonsen) weighin used. The animals were numbere the thigh and lower back was minutes prior to irradiation the t DMSO; 100% DMSO plus MEA

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