

Delayed hypersensitivity in females

The development of allergic contact dermatitis in females during the comparison of two predictive patch tests

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Ten chemically different compounds were applied to the skin of the backs of male and female volunteers to induce delayed hypersensitivity responses. The 10 compounds had a low, moderate, high or unknown allergenic potential and were divided among five test panels (modified Draize) consisting of 150 subjects each. In each panel, 75 subjects (mean age 31 years) were always male prisoners (50 % Caucasians) and the remaining 75 subjects (> 90 % Caucasians) were divided, age-matched free males and females (mean age 24 years). Three compounds, each on different panels, had a significantly higher sensitization rate in females than in free males or total males. The remaining compounds produced sensitization, usually in lower numbers, but females dominated in sensitization rates. Viewed as absolute rates of sensitization for the 10 compounds, females were higher on eight test compounds and tied on two when compared to all males. These studies show that females develop significantly more cell-mediated immune responses to some chemical haptens than men and the data also points to an overall higher rate of hapten recognition. Seven of these 10 compounds were simultaneously tested for their sensitization potential by the modified Maximization method (all males) in order to compare the two tests' ability to disclose potential sensitizers. Female sensitizations on the modified Draize accounted for this test's superiority.

Key words: Dermatitis, contact – hypersensitivity, delayed – sex – skin tests.

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Females appear to develop delayed hypersensitivity to chemical haptens at a greater rate than do males. This difference became apparent as we were making direct comparisons of the Maximization and modified Draize methods of inducing sensitization (Kligman 1966b, Draize et al. 1944).

The Maximization and Draize procedures are the two principal tests used for the so-called predictive patch testing of chemicals or finished products for intended contact with the skin. They have been modified since their introduction (Kligman & Epstein

1975, Marzulli & Maibach 1973, 1974), and we describe here our simultaneous comparison of seven chemical entities on both modified tests. We found our modification of the Draize test to be definitely superior; the Draize test included females and the Maximization tests did not. Draize tests, using both sexes, were run on three additional chemicals and this report presents the male-female sensitization rate for a total of 10 compounds. In addition, we also describe our experiences with the two predictive tests.

Methods

Our modification of the Draize test consisted of 150 subjects, of which 75 were male inmates in a penitentiary and 75 were free subjects in the medical center area. Females comprised one half of this free population. The mean age of both sexes in the free population was 25 years for the males and 26 years for the females. The free population was greater than 90 % Caucasian. The mean age for the inmate population was 30 years, and 50 % were Caucasian. The test chemicals were applied to the same site on the upper back on Monday, Wednesday, and Friday for 3 consecutive weeks. The patches were not removed until the next scheduled patch test replacement day. At the conclusion of this induction phase, a 2-week rest period ensued followed by two consecutive 48-h challenge patches on the same site, but away from the original induction site. Five 150-subject panels were used in the testing of the 10 chemical entities on the modified Draize procedure. Four of these panels tested seven of the compounds that were simultaneously tested on four Maximization panels described below.

Maximization tests were carried out on 25 to 30 inmates having the same mean age and racial composition as the male inmates on the modified Draize test. The Maximization test was conducted according to the recent modification with the exception of the challenge sequence. Our previous experience with sodium lauryl sulphate (SLS) provocative challenges (Kligman 1966a, Kligman & Epstein 1975) has been unreliable in that it may produce false positive responses particularly if the test compound is in a petrolatum vehicle. Since we were evaluating the ability of both tests to induce sensitization by different methods, we elected to challenge the chemicals in the Maximization test by the same newly exaggerated method employed in our modi-

fication of the Draize. The merits of this challenge method will be discussed.

Materials

The adhesive patches were a modification of the Read-Band® (Parke-Davis) specifically constructed for predictive testing. The perforations have been removed from the adhesive mass, and a 2 cm × 2 cm cotton pad is used to hold the test material. These patches are made on specific request and are referred to as the Hilltop Patch. Dermacil® cloth tape (Johnson & Johnson) or Scanpor® (Norgesplaster A/S) paper tape was used over these patches for reinforcement.

Listed below are the chemical entities examined in one or both tests. They satisfy one or more test situations that we feel should be taken into account when evaluating or comparing predictive patch tests:

1. Some test compounds should be included that examine the test's ability to disclose substances that cause contact dermatitis on more than a rare occasion and yet do not constitute a frequent allergen in dermatologic practices. They have little impact on the population as a whole. These compounds are best described as surviving nicely in the market place. The ability of a test to have at least one positive reactor to such an allergen defines the lower limits of discrimination for the test and is helpful in assessing the impact of any new chemical with a similar score and type of exposure. Dichlorophene® and p-chloro-m-xyleneol were chosen as examples in this category.
2. Some compounds to be tested should have been tested previously by other investigators. In addition to the two compounds listed in (1), Captan®, Furacin® (Marzulli & Maibach 1973), and the op-

Table 1. Sensitizations obtained for 10 test substances on this 150-subject, modified Draize test and comparison of these test results with those of the Maximization tests (seven test substances)

Agent	Induction-Elicitation (% concentration)*		Maximi- zation 25-30 subjects	Modified Draize 150 subjects	Literature citations of previous sensitization scores**
Cinnamic alcohol	4 4 alc.	4 4 alc.	0 1	0 4	M - 0/25 Opdyke (1974)
Calcofluor ALF	0.1 .05 aq++	0.1 .05 aq++	0 0	0 18	D - 0/69, 4/56, 16/65 Griffith (1973)
Imidazolidinyl urea (Germall-115)	2 aq	2 aq	0	2	-
Captan	1	1	3	8	D - 9/205 Marzulli & Maibach (1973)
Dichlorophene	5	1	0	1	D - 0/200, 1/200 Marzulli & Maibach (1973)
Hydroxycitronellal	4	4	0	1	M - 0/25 (4 & 12 % induction conc.) Opdyke (1974)
Synthetic beeswax (in a w/o cream vehicle)	12.5	12.5	0	2	M - 0/25, unpublished data from Johnson & Johnson (April 1976)
p-chloro-m-xyleneol	20	5	-	5	D - 0/208, 0/110 Marzulli & Maibach (1973)
Furacin	20	20	-	1	D - 0/99 Marzulli & Maibach (1973)
Chloracetamide	0.5 aq	0.5 aq	-	47	D - 35/205 Marzulli & Maibach (1973)

* All test items in petrolatum alba unless otherwise noted.

** M - Maximization

D - Draize

++ aq - a 0.5 soap solution

3. Some compounds should be known sensitizers and a lack of prior exposure to these a virtual certainty. Chloracetamide® (Marzulli & Maibach 1973) and Calcofluor ALF were chosen for this purpose.
4. This category of test compounds includes substances that have been shown to be responsible for a significant or increasing number of relevant patch tests in clinical material and yet are known to have passed one or more predictive tests either as a single entity or part of a final product formulation. Cinnamic

Table 2. Percentage of females sensitized to test compounds compared to age and race-matched males (free) and total males

Item	Female	%	Male Free Inmate	%	% Total male
Cinnamic alcohol	4/32	12.5	0/43 0/76	0 0	0
Calcofluor	5/32	15.6	6/43 7/75	13.9 9.0	11
Imidazolidinyl urea	1/36	2.7	1/40 0/74	2.5 0	0.8
Captan	5/36	13.7	0/40 3/74	0 4	2.7
Dichlorophene	1/36	2.7	0/40 0/74	0 0	0
Hydroxycitronellal	1/45	2.2	0/31 0/76	0 0	0
Synthetic beeswax	2/47	4.2	0/30 0/70	0 0	0
p-chloro-m-xyleneol	1/33	3	0/42 4/76	0 5.3	3.3
Furacin	0/36	0	0/40 1/74	0 1.3	0.8
Chloracetamide	19/33	57.5	12/42 16/72	28.5 22.2	24.6

alcohol, Hydroxycitronellal, and Imidazolidinyl urea (Germall-115®) were chosen as typical of this category.

5. A commercial cream containing a synthetic beeswax is included as a test allergen because it was examined on both tests during the study and limited commercial use data following the tests were available.

Two compounds, cinnamic alcohol and Calcofluor ALF, were tested in petrolatum and then retested in 70 % ethanol (cinnamic alcohol) and water (Calcofluor ALF).

Results

Maximization and Draize modification. For seven compounds run on both tests the Maximization test showed positive responses for two, one sensitization for cinnamic alcohol (alcohol vehicle) and three sensitizations for Captan. It was ineffectual for disclosing sensitization to Calcofluor ALF, Hydroxycitronellal, Dichlorophene, Germall-115, and the synthetic wax. The Draize modification had at least one sensitization to all seven, and 18 sensitizations to Calcofluor ALF (in water) (Table 1). Neither

the Maximization nor the Draize method showed sensitizations when cinnamic alcohol and Calcofluor were tested in the petrolatum vehicle.

Male-female sensitization. Table 2 shows the percent of females sensitized to the compounds by a modified Draize test in relation to free males, inmates, and total males. Using Fisher's exact test, sensitizations for females were significantly different from that for age-matched free males or total males for cinnamic alcohol, Captan, and Chloracetamide (all P values $< .03$). Rates of sensitization for the remaining compounds were low for both groups, but females were an average of 2.7 % higher (1.7–4.2 %) in sensitization rates than free males for five other compounds tested. Using a greater than 1 % sensitization difference as a win or loss, women were higher in eight and lower in two test compounds when compared to inmates. Compared to all males, women were higher in eight and tied twice. There was no difference in inmates and free male sensitization rates for four compounds, and each group had three wins each for the remaining six compounds.

Double 48-hour challenge: There were a total of 93 allergic responses recorded in all the tests. Thirty-six of these responses were not present at the end of the first 48-h challenge reading (38 %).

Comment

The ability of the sexes to develop cell-mediated responses to chemical haptens has been assumed to be equal. Anytime there has been a sex difference to common allergens, the likelihood of different exposures is usually the answer (Fregert et al. 1969). We found several reports in the literature that applied to our work. A report by Schønning & Hjorth (1969) was of in-

terest because they found that their concentrations for diagnostic patch tests of primula and two paraphenylenediamine derivatives induced more spontaneous flares in women. They explained that women flared because prior exposure had set them up to react 7 to 14 days after the negative diagnostic patch test. This proposal appears strengthened by the fact that women outnumbered men for positive primula reactions at the normal delayed reading time of 48 to 120 h. Assuming an equal sex ratio of tested subjects, a predominance of one over the other would signify an exposure difference. However, a sex preponderance for diagnostic test reactors does not mean the same will exist for spontaneous flares if it can be shown that spontaneous flares can occur more frequently in one sex while the sex ratios for numbers tested and positive diagnostic reactors are equal or favor the other sex. This situation occurred in their spontaneous flares to the two para-derivatives. There were 17 initial male reactors and 14 females. Twenty previously negative females flared, but only seven males showed this sensitization ($P < 0.01$). A case for lopsided prior exposure for females is not evident in the initial 31 positive subjects.

The most obvious source of information on the deliberate sensitization of the sexes would be the reports on well-known experimental sensitizers used on normal controls. Walker et al. (1967, 1969) could show no significant difference with the moderate sensitizer NDMA. Males dominated for DNCB by their method of sensitization, but most other sensitizations have been carried out with concentrations giving such high rates of allergic responses that any differences could be obscured. Other than NDMA, deliberate sensitizations using moderate sensitizers in normal male-female subjects have not been reported. Rea (written communication August 1976) has noted

a significant increase in spontaneous flares to DNCB-DNFB in women. The latest report by the Boston collaborative drug group, concerning adverse cutaneous responses to orally administered drugs in the hospital, reported females had a 50 % higher rate of cutaneous reactions after correcting for hospital admission differences (Arndt & Hershel 1976). This study is only mentioned out of peripheral interest because not all of these reactions could be proven or immunologically defined. The evidence in this report is strong that females are more likely to develop delayed hypersensitivity to some haptens. Our report also shows females are likely to have this ability over a wide range of chemicals.

There is no argument that irritation aids and abets any allergic potential in a chemical. A lower threshold for skin irritation, leading to an increase in sensitization rates for women, should be the first consideration in explaining the differences reported here. Our irritation scores, compiled during the induction phase, showed no differences for the three compounds having significant sensitization rates. Chloracetamide was virtually free of irritation. Bjornberg (1975) concluded that, with few exceptions, there were no statistically significant differences between the sexes for 11 irritants examined. Lanman et al. (1968) found men developed irritation sooner for five test items in one test observation.

The role of estrogen and progesterone levels at various times in the menstrual cycle is unknown regarding their ability to affect the induction of delayed hypersensitivity. If one or the other hormone enhances the induction of delayed hypersensitivity, then most of the women on this test would likely be in the induction portion of the test at some time during this hormonally-enhanced period. The induction period covers 21 days.

The clear superiority of this modified

Draize test over the all male Maximization test is due to the sensitization rates of the females included on the former. Female sensitization rates were significant for three compounds and each compound was on a different panel. We would reject the argument that past exposures for the female panelist influenced sensitization. Cinnamic alcohol, a bellwether in perfume formulations, could be considered in the prior exposure argument, but Captan, predominantly a fungicide, and particularly Chloracetamide, with almost no USA exposure, demonstrate that these rates were not due to past exposure. The idea of prior exposure influencing the final challenge test is rejected unless at least one of the test subjects flares with an allergic response during the induction phase. We feel that, if subjects do not flare in the induction or rest phases and are allergic in the elicitation phase, it is unlikely that exposures other than the recent induction phase influenced the allergic challenge patch. Women were non-significant winners on the optical brightener, Dichlorophene, and a new synthetic wax. Except for the wax, all compounds were selected because they were known to be capable of being more than just a rare sensitizer. An average panel size of only 35 women uncovered eight of the nine potential allergens. An equal number of free males had no responses to six of these nine. In fact, an average of 115 males pointed out this potential in only six of the nine. The synthetic wax, with 4.2 % female responders and no male responders, was evaluated in a small test market in the vehicle used for the sensitization test. Complaints received by the manufacturer were carefully followed up and the allergic reactions documented were subsequently found to be due to this wax. A Maximization test recently failed to disclose sensitizations to this material (Table 1).

We find the latest modification of the

Maximization test on males no better, and possibly inferior, to an all-male modified Draize test having one half of its normal compliment of subjects. It should be pointed out that our comparison of these two tests was really a comparison of the two tests during their induction and rest phases. The challenge for the Maximization test (provocative with sodium lauryl sulphate) differs from the 48- to 72-h single, nonprovocative challenge of the usual modified Draize test. We challenged in both tests by a back-to-back 48-h patch test. This added an extra dimension to the previous Draize challenges. It would also decrease the liability of obtaining a false positive with SLS on the Maximization test, and counterbalance the negative aspect of removing SLS. The 1-hour prechallenge with SLS was originally intended to bring out a timid allergic reaction for whatever reason (Kligman 1966a). The double 48-h patch tests described here have been compared to the SLS provocative challenges on Maximization tests run prior to this study (unpublished observations). Subjects on a Maximization test have been simultaneously challenged by the SLS provocative method and a double 48-h elicitation patch. The second 48-h challenge patch has always overcome the differences noted at 48-h between a truly positive SLS pretreatment challenge patch and a negative non-SLS challenge patch. Of our positives, 38 % were recorded at the end of the second challenge. Undoubtedly responses would have appeared by 96 h had we not undertaken a second challenge; we have never seen delayed reactions prior to this technique at this rate. We understand what the provocative SLS challenge is supposed to accomplish in principle, but the means never justify the end. A 5 % concentration of SLS for 1 h on a patch test site followed by the allergen in a petrolatum base can give misleading results. A control SLS-petrolatum patch is supposed to clarify any

doubtful results, but we have seen the control patches show positive reactions (irritation) while the SLS-allergen in a petrolatum vehicle was negative. If this combination can arise, and we have seen it on more than one occasion, then the opposite response will always have to be viewed with suspicion. A patch test is meant to elicit a response with no other variables than the allergen. Adding a potential irritant to a yes-no test for allergy invites useless positive responses. Historically, the SLS 1-h provocative challenge test has gone from 10 % to 5 %, and now sometimes 2.5 %. This is either a downward trend to zero or an artful interpretation of what each test subject will require to make the reaction just right.

The 150-subject Draize modification panels described here had at least one reaction to all 10 test compounds selected; some of these compounds were not sensitizers on other Maximization or Draize panels previously reported (Table 1). The induction and elicitation concentrations, with rare exceptions, were the same.

The tests reported also point out the potential hazard of clearing a significant allergen because it was examined in the wrong vehicle. All new compounds should be evaluated in more than one vehicle at exaggerated concentrations to reduce this possibility.

These 150-subject panels are 50 subjects less than previously reported for modified Draize tests. Statistically speaking, this reduction is negligible when compared to a 200-subject panel. The major differences are apparent when a panel size is reduced to 100 subjects or increased to 300 subjects. If there are no responders in a panel of 150 or 200 subjects, the maximum permissible percentage of reactions in the population will be 2.0 and 1.5 respectively (95 % likelihood). For 300 subjects, the percent falls to 1; for 100 subjects, it rises to 3 % (Hen-

derson & Riley 1945). None of these percents of maximum possible reactors consoles the cosmetic manufacturer. Statistically, 150 subjects (2 % maximum reactors) fits halfway within this 100–300 subject range with a considerable savings in money and time required to affect each patch test change over the 200-subject test.

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References

- Arndt, K. & Hershel, J. (1976) Rates of cutaneous reactions to drugs – a report from the Boston Collaborative Drug Surveillance Program. *235*, 918–923.
- Bjornberg, A. (1975) Skin reactions to primary irritants in men and women. *Acta Dermatovenereologica* *55*, 191–194.
- Draize, J. H., Woodard, G. & Calvery, H. D. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics* *83*, 377–390.
- Fregert, S., Hjorth, N., Magnusson, B., Bandmann, H. J., Calnan, C. D., Cronin, E., Malten, K., Meneghini, C. L., Pirilä, V. & Wilkinson, D. S. (1969) Epidemiology of contact dermatitis. *Transactions of the St. John's Hospital Dermatological Society* *55*, 17–35.
- Griffith, J. F. (1973) Fluorescent whitening agents – tests for skin sensitization. *Archives of Dermatology* *107*, 728–733.
- Henderson, C. R. & Riley, E. C. (1945) Certain statistical considerations in patch testing. *Journal of Investigative Dermatology* *6*, 227–232.
- Jordan, W. (1974) Unpublished data.
- Kligman, A. M. (1966a) The SLS provocative patch test in allergic contact sensitization. *Journal of Investigative Dermatology* *46*, 573–585.
- Kligman, A. M. (1966b) The identification of contact allergens by human assay. III. The maximization test. A procedure for screening and rating contact sensitizers. *Journal of Investigative Dermatology* *47*, 393–409.
- Kligman, A. M. & Epstein, W. (1975) Updating the maximization test for identifying contact allergens. *Contact Dermatitis* *1*, 231–239.
- Lanman, B., Elvers, W. & Howard, C. (1968) The role of human patch testing in a product development program. *Proceedings Joint Conference on Cosmetic Sciences*, Washington, D.C. 134–145.
- Marzulli, F. N. & Maibach, H. I. (1973) Antimicrobials: Experimental contact sensitization in man. *Journal of the Society of Cosmetic Chemists* *24*, 399–421.
- Marzulli, F. N. & Maibach, H. I. (1974) The use of graded concentrations in studying skin sensitizers: Experimental contact sensitization in man. *Food and Cosmetics Toxicology* *12*, 219–227.
- Opdyke, D. L. J. (1974) Monographs on fragrance raw materials. *Food and Cosmetics Toxicology* *12*, 807–1016.
- Schønning, L. & Hjorth, N. (1969) Sex differences in capacity for sensitization. *Contact Dermatitis Newsletter* *5*, 100.
- Walker, F., Smith, P. & Maibach, H. (1967) Genetic factors in human allergic contact dermatitis. *International Archives of Allergy* *32*, 453–462.
- Walker, F. & Maibach, H. (1969) Sex differences in contact sensitization. *Contact Dermatitis Newsletter* *6*, 122.

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