

Dimethylsulfoxide as a penetrant carrier of allergens through intact human skin

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The penetrant-carrier capacity of dimethylsulfoxide (DMSO) through intact human skin was studied with allergens of varying molecular sizes and compositions. Results showed that only with DMSO 90 per cent and penicillin could positive Prausnitz-Küstner (P-K) reactions be produced. Larger sized allergens in DMSO applied directly over sensitized sites failed to penetrate sufficiently to produce positive P-K reactions. Removal of stratum corneum permitted the production of positive P-K reactions with allergens of all molecular sizes and in simple buffered saline.

The capacity of dimethylsulfoxide (DMSO) to penetrate and carry certain chemicals through intact skin and mucous membrane has been well documented.¹⁻⁴ Studies have included the use of varying concentrations of DMSO with drugs tagged radioactively with carbon 14 as well as with fluorescent dyes^{5, 6} on excised and intact skin.

We have used nonallergic men and have employed immunological methods to demonstrate the passage of allergens with DMSO through intact skin to react at a sensitized site containing the specific reagin, resulting in the typical Prausnitz-Küstner (P-K) reaction. A number of experiments were conducted with various sera and with a number of allergens varying in molecular size from penicillin (molecular weight less than 350) to dog hair and house dust (molecular weight more than 300,000). Penicillin G potassium (Pen. G.K.) as the allergen proved most successful since it could be applied with DMSO at a remote area from the sensitized site, thus eliminating the obscuring effect of DMSO which, in the concentration used, itself often caused redness and urtication.

It had been previously observed that, with the specific reaginic sera, penicillin would flare up the sensitized site by a remote challenge either by scratch or intradermal test or by mucosal (sublingual) application. As little as 10 units of Pen. G.K. injected intradermally at a remote site could elicit full reaction within twenty minutes. This same speed of reaction was noted also with challenge by scratch testing or sublingual application.

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MATERIAL

The dimethylsulfoxide (DMSO) was the experimental drug—grade sterile and pyrogen free (minimum DMSO contents of 99.6 per cent). It was mixed with each allergen in the proportion of nine parts DMSO to one part allergen, making a 90 per cent solution of the solvent.

Allergens

Penicillin. Penicillin G potassium (Pen. G.K.) was freshly constituted with distilled water at the start of each experiment and was further diluted with normal saline buffered to pH 7.4.

House dust and dog hair. These allergens were highly concentrated extracts currently in clinical use. They caused strong positive skin test reactions on the donor of the serum.

Sericin. The allergen, sericin, was prepared by soaking laboratory-grown silk cocoons in boiling water which dissolved the soluble "cement." This solution of sericin was converted into a solid state by lyophilization. A solution of the powdered material was highly allergenic on allergic serum donors sensitive to silk.

Timothy pollen fractions. Between the highly purified, highly allergenic fraction (D.E.A.E. II) and the dializable haptenic fraction (W.S.T. II),* the haptenic fraction elicited the less marked skin reaction on passively sensitized sites.

Castor bean extract. A highly purified, water-soluble fraction of castor bean extract was used.† Direct testing on the sensitive serum donor gave a strong positive reaction with an allergen dilution of 10^{-8} .

Sera

The sera for passive sensitization were all collected and stored at -20° C. until used. The titers were quantitated by passive transfer in serial dilutions to the skin of human volunteers and to the skin of monkeys.^{10, 11} The sera varied in the titers of reagin for several allergens, even within a single sample. For example, in one serum the reagin for penicillin was detectable to a dilution of 1:32, for house dust to a dilution of 1:256, and for dog hair to a dilution of 1:512.¹¹

Recipients

These comprised a number of nonallergic men including the two authors who themselves provided the added advantage of a long period of self-observation.

METHODS

The sensitized sites were made in most experiments on the flexor surface of the arm and forearm by injecting 0.10 ml. of the sera intracutaneously; both the place and the direction of the deposit of serum was marked.

The area for the remote challenge with the mixture of allergen and DMSO

*Obtained from Dr. Arthur Malley.^{7, 8}

†Provided by Dr. Laurence Layton of Albany, Calif.⁹

was the flank below the axilla. This mixture in the amount of 0.1 ml. was applied in the center of a 25 square centimeter area and gently spread to the periphery. Then with as much uniformity of pressure as possible, the entire area was rubbed ten times with the head of the plunger of a tuberculin syringe. No abrasion or urticarial streaking was noted. Thus, when urtication from DMSO occurred it was quite uniform. Often there was a spread of this urtication beyond the 25 square centimeters.

The sensitized sites were observed for any reaction for several hours before negative results were recorded. In the case of the author-recipients this observation was possible for a period of more than 12 hours. However, one hour was arbitrarily accepted for a negative report. In those cases of negative results from DMSO and allergens and where direct challenge was performed at the sensitized site, the challenge was done either by scratch test or by intradermal injection of 0.02 ml., with an effort to deposit the allergen in the direction and at the site of the serum.

OBSERVATIONS

Local skin effects of DMSO varied with individuals. Some had minimal or no subjective or objective reactions, while others complained of marked pruritus with minimal or no visible effect. Several had mild reddening and urticating reactions. One author-recipient (H. W.) consistently had a pruritic, erythematous, and urticating reaction which generally subsided in several hours, while another recipient (F. P.) had little or no redness and minimal itching.

Undesired systemic effects were rare. None of the subjects detected the familiar "oyster taste" from the small amount of DMSO applied. One recipient (F. P.) developed a mild pruritic dermatitis five days after the application of DMSO with penicillin. Another recipient (H. W.) developed generalized urticaria on the extremities and trunk after one of the applications of DMSO and penicillin. This rapidly disappeared after 4.0 mg. of Chlortrimeton was administered orally. Since H. W. gave consistently negative reactions on direct intradermal testing at the control site, with Pen. G.K. in amounts of 100 units, both before and after this episode, it was thought that this generalized urticaria was similar to the occasional reaction reported with the use of DMSO over large areas.

CONCLUSIONS

A series of six experiments is summarized in Table I. The conclusions which follow are based on the observations made in these experiments.

1. Employment of the Prausnitz-Küstner (P-K) reaction offered a method for demonstrating the penetrant-carrying capacity of DMSO through intact human skin.

2. An allergen of small molecular weight such as penicillin G. potassium mixed with DMSO 90 per cent was readily carried through intact skin (Experiments A, B, and E).

3. Allergens of large molecular weight (3,000 and larger) could not be shown to penetrate skin with DMSO within the prescribed hour's time, even

Table I. *Summary of experiments*

<i>Experiment</i>	<i>Objectives (questions)</i>	<i>Allergens</i>	<i>Procedures</i>	<i>Observations</i>
A.	What concentrations of DMSO will carry allergens through intact skin of flank to light up remote sensitized sites on arm and forearm?	Pen. G.K., 1,000 units	Buffered saline DMSO 25% DMSO 50% DMSO 90%	No reaction at arm sites No reaction at arm sites No reaction at arm sites Positive reaction at arm sites in 40 minutes
		House dust Dog hair	DMSO 90%	No reaction at arm sites (Direct scratch test at sensitized sites gave wheal and flare in 20 minutes)
B.	Will allergens of varying molecular weights vary in penetration with DMSO on intact skin?	Pen. G.K. Celery Buckwheat Castor bean Cottonseed	Each with DMSO 90% challenged first on flank then directly by scratch over sensitized arm sites	Penicillin in DMSO reacted from flank challenge on 3 of 4 subjects in 40 minutes None of larger allergens reacted from flank challenge All sites reacted to all allergens on direct challenge by scratch test
C.	Do other rubefaciants have penetrant-carrying capacities within the prescribed 60 minute period?	Pen. G.K., 1,000 units	Trafuryl 5% in acetone (tetrahydrofurfuryl ester of nicotinic acid) Oil of mustard 10% in acetone	Marked redness and itching of flank from both but no flare of sensitized arm sites Later challenge with penicillin sublingually flared arm sites in 20 minutes
D.	Is there a binding or denaturing effect of DMSO on allergens? (Allergens of relatively small size and sera with high reagin titers were used)	Castor bean	DMSO 90% and allergen were applied to the flank	Marked redness of flank but no flare of sensitized sites
		Sericin (silk)	Portions of the same mixtures after incubation at 37° C. for 30 minutes were applied later by scratch to the sensitized sites	Wheal and flare in 20 minutes at scratch site indicating no binding or denaturation of allergens by DMSO
E.	Simultaneous testing with purified timothy pollen fractions and with penicillin in DMSO	Timothy pollen fractions: D. E. A. E. II and W. S. T. II	Each allergen in 90% DMSO was first applied to the flanks of the two author-subjects Each mixture of timothy pollen fractions after incubation at 37° C. for 30 minutes was later applied to scratch tests directly over sensitized sites on the arm	Timothy pollen fractions did not flare remote arm sites, while penicillin flared all specific arm sites Direct scratch test challenge with pollen fractions in DMSO gave wheal and flare in 20 minutes

Table I. *Cont'd*

<i>Experiment</i>	<i>Objectives (questions)</i>	<i>Allergens</i>	<i>Procedures</i>	<i>Observations</i>
		Pen. G.K., 1,000 units	Powdered penicillin was placed under the tongue 90 minutes after allergen was first applied	Sublingual challenge with penicillin on subject F.P. (whose flank did not redden and whose remote arm sites showed small wheals) resulted in increase wheal and flare
F.	What part does the stratum corneum play as a barrier to the rapid penetration of allergens of varying sizes in DMSO and in buffered saline?	Pen. G.K.	Penicillin in saline applied to the flank area stripped of stratum corneum	Wheal and flare of remote sensitized sites in 20 minutes
		House dust	Large molecular size allergens in saline applied to area over sensitized sites stripped of stratum corneum	Wheal and flare of sensitized site after 20 minutes
			Large molecular size allergen in DMSO applied to sensitized sites but with stratum corneum intact	No reaction other than mild redness due to the DMSO

when the challenges were made directly to the intact skin over the sensitized sites (Experiments A, B, D, E, and F).

4. Inflammatory reaction was not the basis for such penetrant-carrier effect of DMSO, as shown by lack of such effect with the use of other rubifacient agents and within the hour's time (Experiment C).

5. The lack of rubifacient effect of DMSO occurred in recipients who also showed less and, in one case, no absorption of the allergen, Pen. G.K. (Experiment B). However, even without redness, some remote P-K reaction was elicited, suggesting that the inflammatory effect is merely the result of an individual skin response and is not a necessary part of the mechanism by which DMSO acts as a penetrant-carrier (Experiment E).

6. DMSO, 90 per cent, incubated with a number of the allergens at 37° C. for 30 minutes did not bind or denature or in any other way appear to alter the allergens' activity, as shown by strong positive reaction upon scratch test challenge with such mixtures to the sensitized sites (Experiments D, E, and F).

7. The time required for a remote P-K reaction after challenge with penicillin in DMSO on intact skin was 40 minutes. This was in contrast to the more rapid rate of reaction after challenges with aqueous extracts of allergens by scratch, intradermal, or mucosal routes, which was usually complete within 20 minutes.

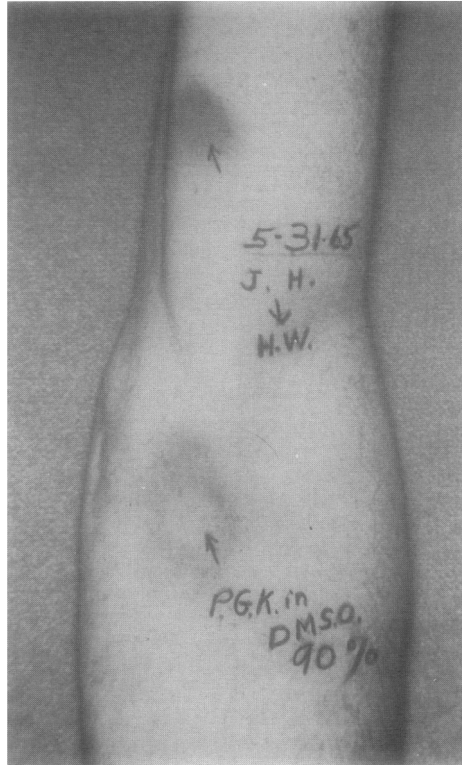


Fig. 1

Indirect Prausnitz-Küstner (P-K) reactions at sensitized sites on arm of recipient *H. W.* forty minutes after challenge to flank with *Pen. G.K.*, 1,000 units, in *DMSO* 90 per cent (Experiment A).

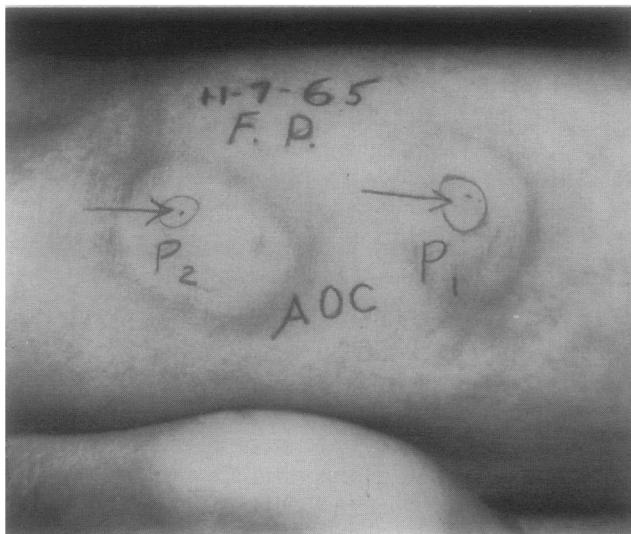


Fig. 2

Small circles, P_1 and P_2 , are representative of the extent of indirect P-K reaction at sensitized sites on the arm of recipient *F. P.* forty minutes after remote challenge to flank with *Pen. G.K.*, 1,000 units, in *DMSO* 90 per cent, which did not produce redness or urtication. Large surrounding wheal resulted from sublingual challenge with minute amount of powdered *Pen. G.K.*; it developed in twenty minutes (Experiment E).

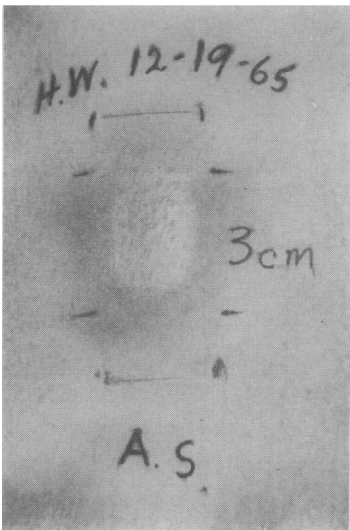
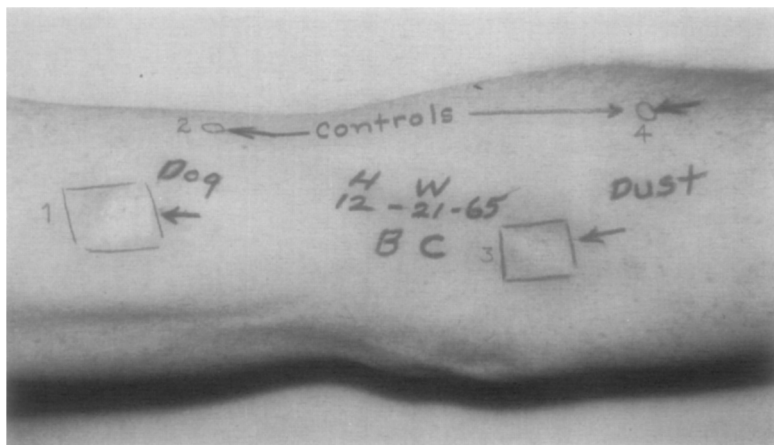


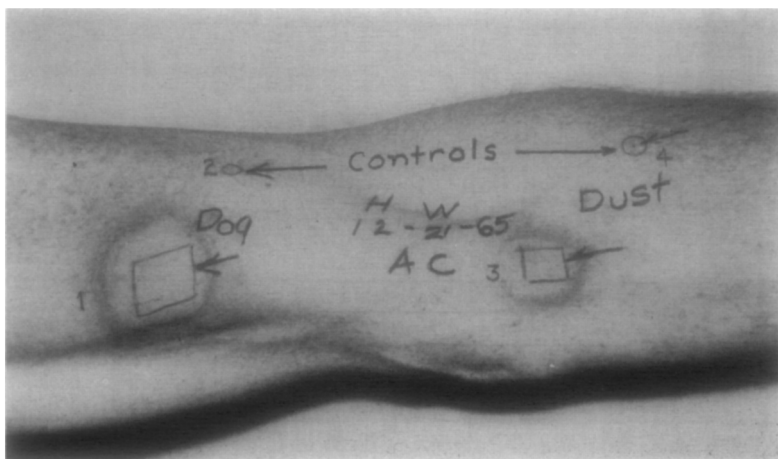
Fig. 3
Site on flank of recipient *H. W.* stripped of stratum corneum showing glistening layer, before application of Pen. G.K., 200 units, in buffered saline (Experiment F).

Table II. *Passive transfer testing on four volunteers sensitized with seven sera and challenged first with penicillin G potassium and larger molecular size allergens in DMSO—on flank and then by direct scratch test of sensitized sites (Experiment B)*

Serum donors and allergens	Recipients							
	J. H.		H. J.		F. B.		G. C.	
	Remote with DMSO	Direct scratch test	Remote with DMSO	Direct scratch test	Remote with DMSO	Direct scratch test	Remote with DMSO	Direct scratch test
J. H. (Penicillin)	++	++++	—	++++	++	+++	++	+++
P. H. (Celery, buckwheat)	—	+++	—	++++	—	++	—	+++
V. McM. (Caster bean)	—	+++	—	+++	±	++++	—	++++
R. McQ. (Caster bean)	—	++	—	++	±	++	±	++
L. H. (Cotton-seed)	—	++++	—	++++	—	+++	±	++++
B. R. (Celery)	—	++	—	n.c.	—	+++	—	++
M. R. (Penicillin)	++	+++	—	+++	+++	+++	++	+++

**Fig. 4**

Sensitized sites 1 and 3 on arm and forearm of recipient *H. W.* stripped of stratum corneum and showing glistening layer before application of the allergens (dog hair and house dust) in buffered saline. Sites 2 and 4 act as controls (Experiment F).

**Fig. 5**

Sensitized sites 1 and 3 showing direct P-K reactions after removal of stratum corneum and application of allergens in buffered saline. Negative controls are sensitized sites 2 and 4 challenged with the same allergens but with the stratum corneum intact (Experiment F).

8. Removal of the stratum corneum by stripping at a remote area and the application of penicillin in buffered saline, as well as stripping over sensitized sites and the application of larger allergens in saline, resulted in a positive P-K reaction at a speed (20 minutes) comparable to direct intradermal challenge. This was in contrast to the inability of larger molecular size allergens in DMSO to penetrate skin, even when applied directly over sensitized sites but with the stratum corneum still intact. (Experiment F).

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