Environmental Health Perspectives
Vol. 70, pp. 229-237, 1986

Induction of Contact Dermatitis in Guinea Pigs by Quaternary Ammonium Compounds: The Mechanism of Antigen Formation

by Karin U. Schallreuter,* Karl Heinz Schulz,† and John M. Wood*

Eight quaternary ammonium compounds were tested for their ability to induce contact dermatitis in guinea pigs by using a modified Freund's complete adjuvant test together with the guinea pig maximization test. Only two quaternary ammonium salts of the eight tested could be designated as strong allergens. These two active substances were shown to be capable of stable association with membrane lipids in forming immunogenic complexes.

This surface complexation phenomenon was confirmed by using a spin-labeled quaternary ammonium salt which competed for binding sites at the surface of epidermal cells *in vivo*. Electron spin resonance was used to demonstrate that stable "ion-pairs" are formed between binding sites and the two allergenic preservatives. Furthermore, information was obtained on the kinetics of immunogenic complex formation as well as on the position and orientation of the quaternary ammonium ion at the cell surface.

The initial contact reaction between allergens and specific molecules in membranes which combine to form immunogenic complexes in the skin has escaped definition (*1*). This reaction is complicated by the large number of chemical substances which are capable of causing both irritant and allergic contact dermatitis. It is clear that such delayed hypersensitivity reactions can be induced by a variety of chemical mechanisms. Reactive compounds include: those which form covalent complexes with cell surface proteins (*1*), metal ions which form a variety of coordination complexes with cell surface ligands (*2*), compounds which form hydrogen bonds with surface proteins to bring about structural alterations through conformational changes (*3*), and simple cations which are able to form "ion-pairs" with negatively charged binding sites. It is apparent that each of the above mechanisms are general in nature and should be expected to occur with functional groups on all membrane proteins inclusive of those specific proteins which form antigenic complexes. Much of the research performed to date in experimental animals has involved the use of haptens which react covalently with cells in the epidermis, or with metal ions such as nickel and chromium, forming a large number of coordination complexes with cell surface ligands. The lack of specificity of most allergens in their reactivity with all proteins makes the search for specific immunogenic complexes formed by the initial contact interaction similar to the proverbial search for a needle in a haystack (*4*). Even the application of radioisotopic methods has failed thus far.

The quaternary ammonium compounds (quats) as possible inducers of delayed contact hypersensitivity were chosen for three reasons: these substances are in widespread use as preservatives and disinfectants, and they are often applied to the skin directly in cosmetics and in medications; quats have a uniform structure with a single active site composed of a quaternary ammonium cation which can form ion-pairs with negatively charged groups; and the induction of allergic contact dermatitis by quats remained controversial. Some reports indicated that quats were allergens or irritants (*5–16*) while others were not.

We present here a systematic study of the ability of a homologous series of quats to cause delayed hypersensitivity reactions in guinea pigs to determine which structural features are important to this allergenicity. Taking advantage of the specificity of this reaction by using a spin-labeled quat we were able to identify the specific location, orientation, and dynamics of this binding site–allergen interaction in guinea pig skin. To our

*Departments of Dermatology and Biochemistry, University of Minnesota, Minneapolis, MN 55455 USA.
†Department of Dermatology, Division of Allergology and Occupational Dermatoses, University of Hamburg, West Germany.

knowledge this approach represents the first specific recognition of the initial binding site for a contact hypersensitivity reaction in the epidermis.

Materials and Methods

Chemicals

The following hydrophilic quats were chosen for this study: quat 1 (acylaminopropyldimethyl-2-hydroxyethyl ammonium chloride); quat 3 (alkylbenzyldi-2-hydroxyethyl ammonium chloride); quat 72 (alkyldipolyethoxymethyl ammonium chloride). Hydrophobic quats selected for this study included: quat 4 (alkylbenzyldimethyl ammonium chloride); quat 8 (alkyldimethylethylbenzyl ammonium chloride); quat 16 (aryltrimethyl-ammonium chloride); quat 20 (benzyldimethyl-2-methyl-4(1,1,3,3, tetramethylbutylphenoxyethoxyethyl ammonium chloride); quat 42 (alkylpyridinium chloride). Quats were obtained from Aldrich Chemical Company, Milwaukee, except for quats 16 and 20, which were obtained from Lonza Company, New Jersey.

Chemical Synthesis and Characterization of Spin-Labeled Quat

The spin-labeled quat was synthesized by reacting 4-bromoacetamido-2,2,6,6, tetramethylpiperidine-*N*-oxyl with dimethyl benzylamine.

Preparation of 4-Bromoacetamido-2,2,6,6,-tetramethylpiperidine-N-oxyl. Following the procedure of Weaver and Whaley (*17*), 1.0 g (6.3 mmole) crude 4-amino-2,2,6,6,-tetramethylpiperidine-*N*-oxyl and 10 mL reagent grade dichloroethylene was placed in a 25-

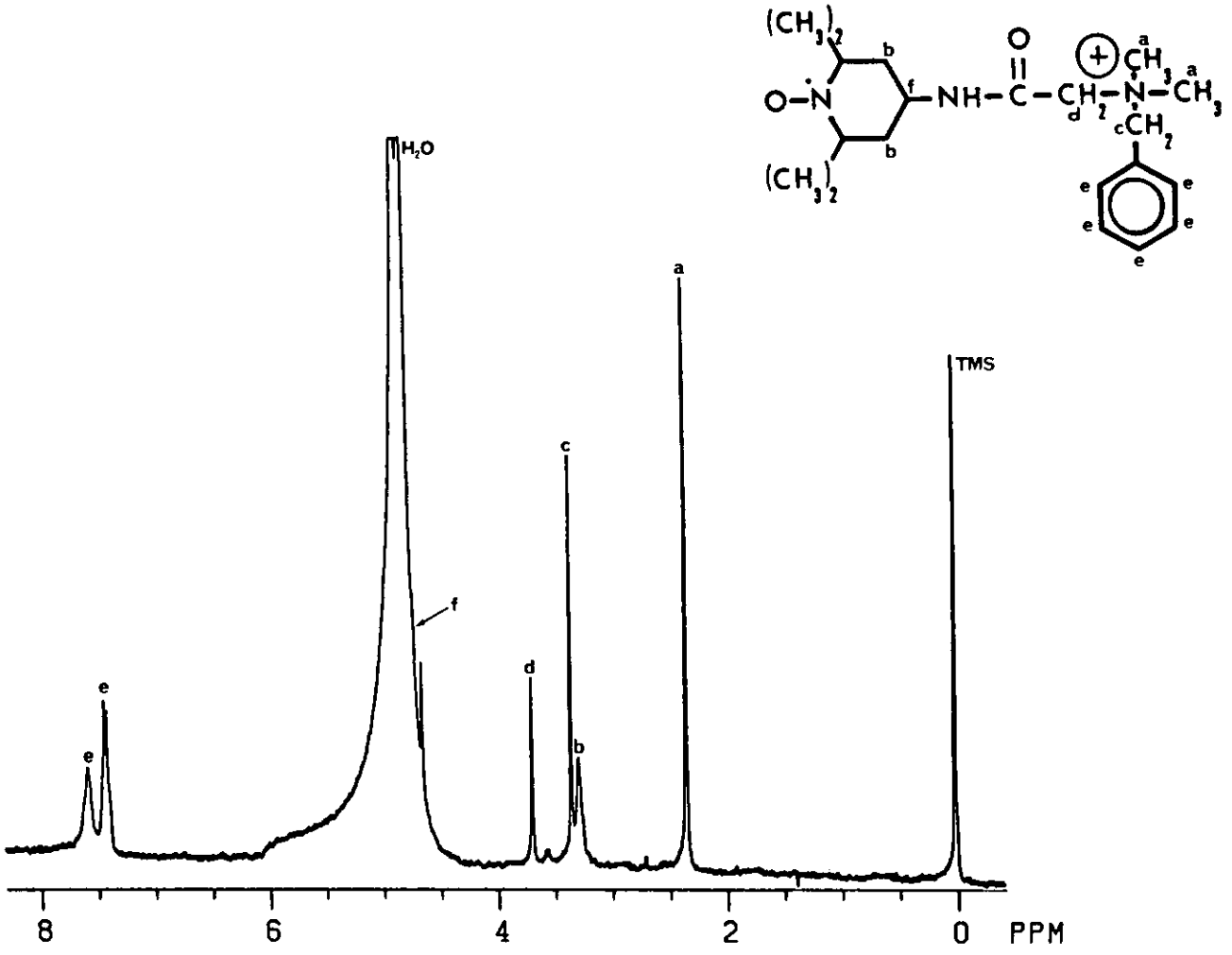


FIGURE 1. 360-MHz ^{1}H-NMR spectrum of spin-labeled quat in D_2O referenced to Tms (0 ppm). The following assignments are supportive of the molecular structure. Two methyl groups on the quaternary ammonium nitrogen at $\delta = 2.34$, benzyl group in quat nitrogen $\delta = 3.34$, methylene group adjacent to the carbonyl $\delta = 3.69$, broadened methylene groups on spin label at $\alpha = 3.278$, phenyl protons at $\delta = 7.56$, 7.42 and 7.40. The proton adjacent to the amido group on the spin label is hidden under the water peak at $\delta = 4.79$, but was resolved as a multiplet in methanol at $\delta = 4.57$. Reduction of the nitroxide radical with dithionite allowed for the resolution of the four methyl groups in the spin label at $\delta = 1.33$ and 1.35. These methyl resonances are not detected in the free radical due to their proximity to the nitroxide paramagnetic center.

mL, two-necked, round-bottomed flask fitted with a dropping funnel, magnetic stir bar, and drying tube. 0.15-g (0.28 mL, 3.2 mmole) portion of practical grade bromoacetylbromide dissolved in 5 mL Ch_2Cl_2 was added dropwise over a 15-min period to the stirred reaction mixture which was maintained at − 10°C. After the addition of acid bromide was complete, the temperature was raised to 25°C, and the reaction stirred for 15 min. The reaction mixture was extracted with three 10-mL portions of 5% HCl. The aqueous extracts were combined and extracted with two 10-mL portions of CH_2Cl_2 which were combined with the previous organic phase. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was recrystallized from benzene-hexane to give orange needles. Analysis: calculated for $C_{11}H_{20}N_2$: C, 45.2%; H, 6.85%; N, 9.5%. Found: C, 46%; H, 6.79%; N, 9.44%; m.p. 123–124°C. Molecular ion in mass spectrometer, calculated: 292; observed: 292.

Preparation of Spin-Labeled Quat. 1.0-g portion of dimethylbenzlamine (Aldrich) was dissolved in 50 mL of dry methanol, and 0.8 g 4-bromoacetamido 2,2,6,6,piperidine-*N*-oxyl was added. The reaction mixture was refluxed for 5 hr, cooled down, and 250 mL of dry diethyl ether added. Pale yellow crystals were formed immediately. They were filtered off, washed with diethyl ether and dried over P_2O_5. The 360-MHz ^1H nuclear magnetic resonance spectrum of this spin-labeled quat is presented in Figure 1. This spectrum confirms the synthesis of acetamido-2,2,6,6-tetramethylpiperidine-*N*-oxyl-benzyldimethyl ammonium bromide. Electron spin resonance experiments were performed on a Varian E_4 spectrometer at 25°C.

The EPR spectrum of this spin-labeled quat gave a_N values of 17.2 gauss as compared with 17.0 gauss for the free reagent, 4-bromoacetamido-2,2,6,6-tetramethylpiperidine-*N*-oxyl (ATEMPO). Line widths of 1.87, 1.87, and 2.20 gauss were recorded with the low field line being broadest. This low field line decreased in intensity and broadened further upon complexation to cell surfaces.

Animals

Female albino guinea pigs (nonpregnant), under 3 months of age with a body weight of approximately 400 g, were chosen for both the toxicity and contact hypersensitivity studies performed herein. Pirlbright white, and compatible white guinea pigs were from Camm Research, New Jersey. Delayed hypersensitivity reactions were tested immunologically by using the modified guinea pig maximization test (GPMT) of Magnusson and Kligman (*18*) and the Freund's complete adjuvant test (FCAT) as modified by Van der Walle (*19*).

GPMT. The GPMT protocols are as follows.

Day 1: intracutaneous injection of 0.1 mL of the test substance in isotonic saline with Freund's complete adjuvant (1:1) on the shaved part of the guinea pig flank (approx. 6 x 4 cm).

Day 7: epicutaneous occlusive application for 48 hr with 0.3 mL of test substance in methyl cellosolve, water, Tween 80 (180/180/40) v/v.

Day 21: epicutaneous open challenge with 0.05 mL of test substance in water at the shaved ventral side of the animal. The response was monitored after 24 and 48 hr next to a control group of 10 animals.

FCAT. The protocols for the modified FCAT are as follows.

Days 1, 5, and 9: intracutaneous injection of 0.1 mL test substance in a mixture of isotonic saline and Freund's complete adjuvant (1:1) on the shaved shoulder of the guinea pig (approximately 8 x 4 cm).

Day 21: epicutaneous application of 0.5 mL of test substance in methyl cellosolve, water, Tween 80 (180:180:40) v/v on the ventral side of each animal. The response was determined after 24 and 48 hr. Ten control animals were treated in the same way without test substance.

Response Criteria. The following criteria for skin reactions were established:

ϕ	=	0	=	no reaction
(+)	=	0.5	=	slight macular erythema
+	=	1.0	=	macular erythema and edema
+/++	=	1.5	=	intermediate erythematous papule
++	=	2.0	=	erythema with induration and infiltration
++/+++	=	2.5	=	severe erythematous papule with induration and infiltration
+++	=	3.0	=	severe plaque-like erythema, with induration, infiltration and crusts

From these observations a mean response (mR) was determined as the ratio of the total response over the number of animals tested, i.e.,

$$\text{mR} = \frac{\text{sum of all positive reactions}}{\text{total number of animals tested}}$$

Strong allergens were classified by a mR greater than 1.0. Weak allergens had a mR of less than 1.0. Also the total number of positive reactions for the whole group of animals tested was included in this analysis.

Concentrations of test substances for delayed contact hypersensitivity experiments were previously ascertained by both intracutaneous and epicutaneous applications to determine irritative toxicity. These results were obtained with groups of three animals at 24 and 48 hr, respectively. In all cases nonirritating concentrations were chosen for these delayed contact hypersensitivity studies.

Histology

Incisional biopsies were taken from test and control animals. These were fixed in formaldehyde, and hematoxylin and eosin (H & E) staining was used for microscopic examinations.

Spin-Labeling Experiments

The spin-labeled quat was tested for its mean response using the FCAT. mR values of 1.0 and 0.6 were found with 0.3% and 0.03% epicutaneous application, respectively. Based on this result, the spin-labeled quat was assessed to be of intermediate allergenicity. For our electron spin resonance experiments, 0.05 mL of spin-label (5% solution) was applied epicutaneously to shaved guinea pigs in a (1:1) solution of ethanol-water. This concentrated solution was chosen to ensure that binding sites at cell surfaces are saturated in the short duration for exposure to spin-labeled quat. Punch biopsies (4 mm) were taken immediately after evaporating the solvent from the surface of the skin. The area covered with spin-label was approximately 1.0 cm in diameter. Superficially bound spin-label was washed from each biopsy with isotonic saline for 5 min. The tissue biopsy was then placed directly in the EPR cell, and the fate of the spin-label was followed with time until only residual bound nitroxide radical signal remained. Tissues with bound spin-label were incubated with 1% solutions of quats 16 and 20 for exchange reactions. After almost quantitative exchange was accomplished, any residual complexed spin-label was removed from biopsies by incubation for 5 min in 80% trichloracetic acid.

Results

Initially an investigation was made of the relationship between structure and function in the allergenicity of a homologous series of quats. This was accomplished by using the Freund's complete adjuvant test (FCAT) and the guinea pig maximization test (GPMT) with groups of 10 and 20 animals, respectively. The results of the FCAT are presented in Table 1, which shows the magnitude of the mean response to six quats after 24 and 48 hr following epicutaneous application of each test substance. The different test concentrations were established after considering the irritative toxicity tests described in Methods. GPMT tests were performed on only three quats with quat 1 being duplicated by both FCAT and GPMT methods. This was done to ensure the efficacy of the two immunological procedures (Table 2). Histological examinations of the induced skin reactions confirmed the cellular pattern and intracellular edema which is suggestive of acute allergic contact dermatitis. The data show that only quats 16 and 20 functioned as strong allergens. These two compounds are structurally similar in having a single long hydrocarbon tail attached to the quaternary ammonium ion with the other three alkyl groups being comparatively small in size (i.e., methyl and benzyl). Quats with small hydrophobic groups in each of the four substitution positions were found to be weak allergens, and those with hydrophilic groups were not active in either the FCAT or GPMT tests. The general formula for the homologous series of quats is shown as I; quat 20, which shows strong allergenic properties, has the structure II.

The importance of the central quaternary ammonium nitrogen atom in allergenicity was established by comparing the abilities of both carbon-substituted analogs (i.e., aryl-*tert*-butyl) and silicon-substituted (i.e., aryl-*tert*-silyl) molecules as allergens in the delayed contact hypersensitivity reaction (FCAT) (Fig. 2). Of the three structurally similar molecules, only the quat caused contact hypersensitivity. These experiments show unambiguously that the quaternary ammonium cation is the functional group which reacts with binding sites on proteins at the cell surface. These initial experiments indicated the importance of quat-lipid interactions in presenting the quaternary ammonium ion to those specific negatively charged surface groups to form stable antigens.

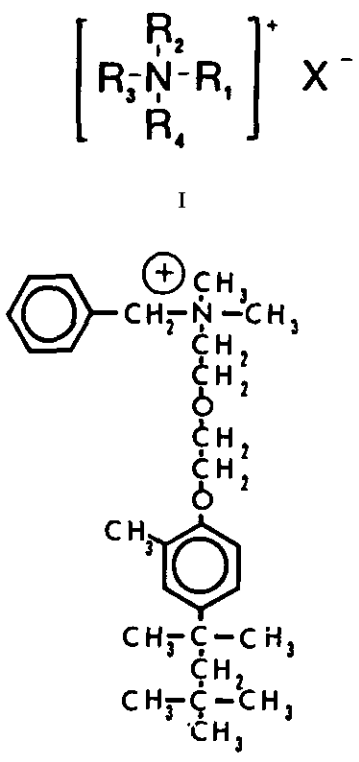
To test this hypothesis of stable "ion-pair" formation through lipid partitioning, we synthesized an analogous spin-labeled quat and showed that it was of intermediate allergenicity in the FCAT test. The need to design a label of intermediate allergenicity is apparent, because stronger allergens would, by definition, have too much thermodynamic stability to be competitively displaced by quats 16 and 20. Therefore, we could take advantage of this spin-labeled quat to examine the specificity of the binding site-strong allergen interaction. The spin-label was applied epicutaneously to guinea pig skin, and 4 mm punch biopsies were taken at different times in order to follow the dynamics for quat-epidermis complexation. Extraneous spin-label was washed from each biopsy with isotonic saline and the epidermis was monitored by electron spin resonance spectroscopy (EPR). It was found that guinea pig epidermis contains an extremely active nitroxide reductase which reduces uncomplexed spin-label to give a diamagnetic product

Table 1. Results of the FCAT test.

	Concentration, %		24 hr		48 hr	
Substance	Intracutaneous application	Epicutaneous application	Mean response	Amimals responding positively	Mean response	Animals responding positively
Quat 16	0.01	0.1	1.7	10/10	1.1	10/10
Quat 20	0.03	0.3	1.5	10/10	0.65	8/10
Quat 72	0.3	3.0	0.9	10/10	0.75	10/10
Quat 8	0.03	0.3	0.2	3/10	0.2	3/10
Quat 1	0.1	3.0	0.2	3/10	0.1	2/10
Quat 3	0.03	1.1	0.15	3/10	0.1	2/10

Table 2. Results of the GPMT test.

Substance	Concentration, %		24 hr		48 hr	
	Intracutaneous application	Epicutaneous application	Mean response	Animals responding positively	Mean response	Animals responding positively
Quat 1	0.1	0.3	0.45	15/20	0.5	13/20
Quat 42	0.03	0.1	0.45	15/20	0.2	7/20
Quat 4	0.1	0.1	0.1	4/20	0.05	2/20



I

II
Quat 20

FIGURE 2a. General structure of quaternary ammonium salts with R_1–R_4 representing the four alkyl substituents and X^- representing the counter anion (e.g. Cl^-, Br^-, etc.), and the structure of the strong allergen quat 20.

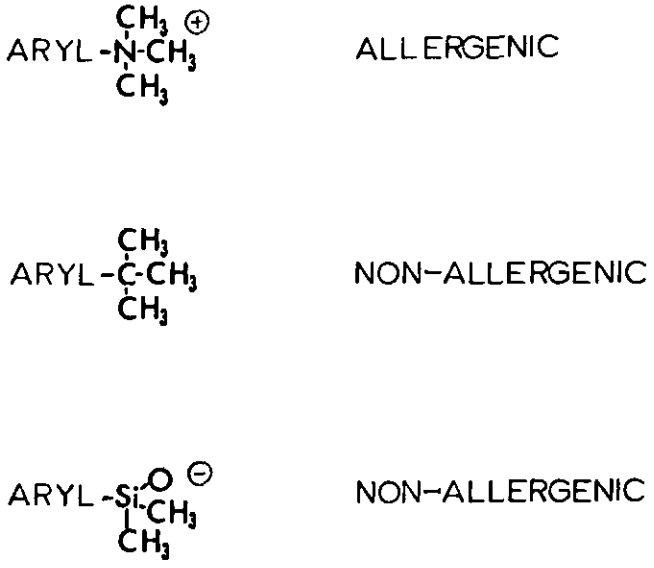


FIGURE 2b. The structures of positive (quat), neutral (*tert*-butyl), and negative (*tert*-silyl) structural analogs tested for their allergenicity in the FCAT test.

which no longer contributes to the EPR spectrum (Fig. 3*a*). Therefore only complexed spin-label, which is surface-bound and inaccessible to the active site of the enzyme, remains to be examined spectroscopically (Fig. 3*b*). This bound nitroxide radical signal has spectral parameters consistent with the attachment of the label at the surface of a macromolecule with some freedom for rotation of the nitroxide functional group from its point of attachment presumably at the surface of a membrane protein (*21,22*). There was no evidence for strong immobilization of the free radical due to membrane lipid or membrane protein partitioning, proving that the receptor site for ion-pairing is located close to the outer polar groups of the lipid bilayer (*23,24*).

Next, we performed an experiment to determine whether the reduction of the free spin-label in epidermal cells occurs through enzymatic reduction or through nonspecific reduction with endogenous reducing agents such as reduced glutathione, etc. In this experiment guinea pig skin was first treated with dinitrochlorobenzene (DNCB), which is not only allergenic but also reacts covalently with all available nucleophilic sites on proteins (i.e., lysine, tyrosine, cysteine). This pretreatment with DNCB was shown to inhibit the nitroxide reduction almost totally (Fig. 4). The residual slow reduction of nitroxide is probably due to nonspecific chemical reduction of the nitroxide radical. Subsequent experiments identified the enzyme as a membrane-associated thioredoxin reductase (*25*).

The experiments described above show that complexation of spin-labeled quat with surface-binding sites occurs more rapidly than the rate of enzymatic reduction of free spin-label in guinea pig epidermis. This reaction is so rapid that it is probably controlled only by the movement of the spin-label through the outer horny layer of the skin.

In the next series of experiments we examined the ionic nature of the spin-label binding site interaction by competitively exchanging this weaker allergen from surface receptors with the two stronger allergens quats 16 and 20. Since these two quats are expected to form ion-pairs which are thermodynamically more stable than

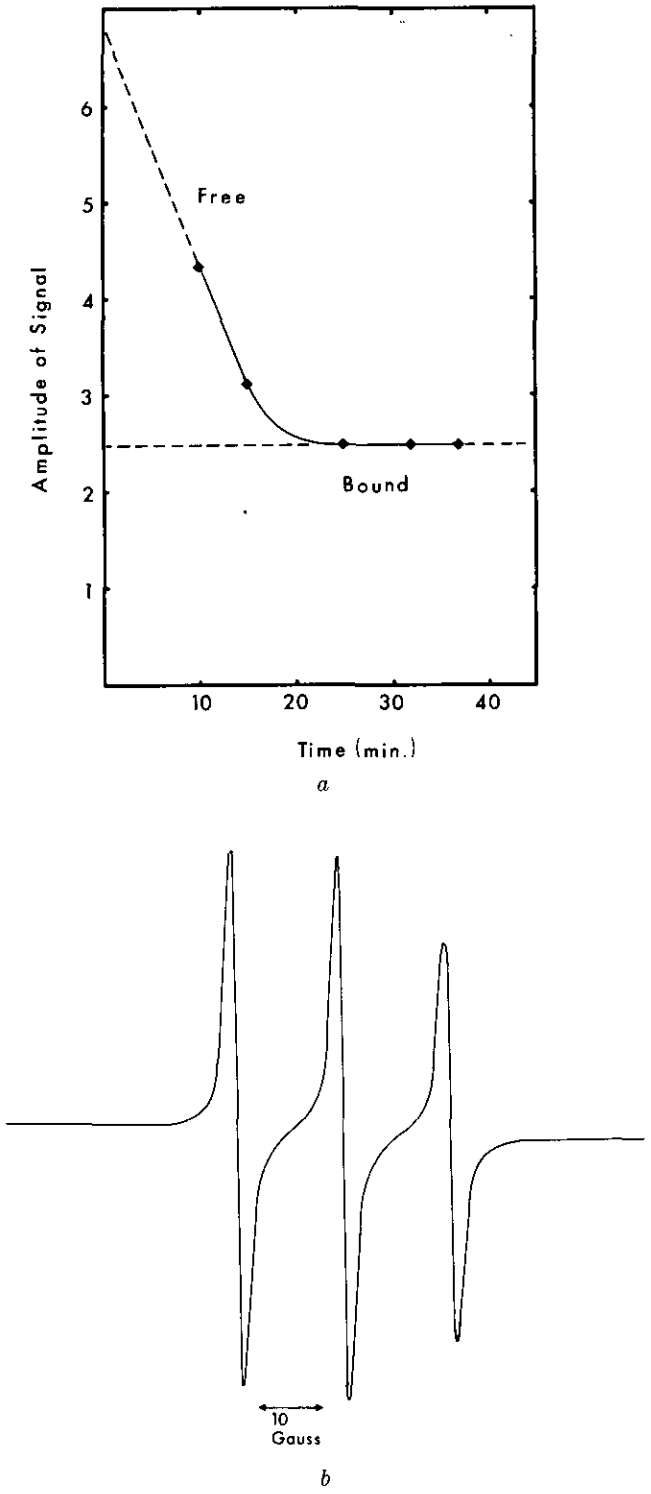


FIGURE 3. (*a*) Enzymatic reduction of free spin-labeled quat by guinea pig epidermis *in vivo*, the residual signal represents bound spin-labeled quat;(*b*) EPR spectrum of bound-spin labeled quat on guinea pig epidermis. This bound EPR spectrum shows line broadening from 11 gauss (free spin-label) to 18 gauss (bound spin-label) in the central resonance. The low field line is broadened and decreases in amplitude which is indicative of surface complexation with some freedom for rotation of the spin-labeled group.

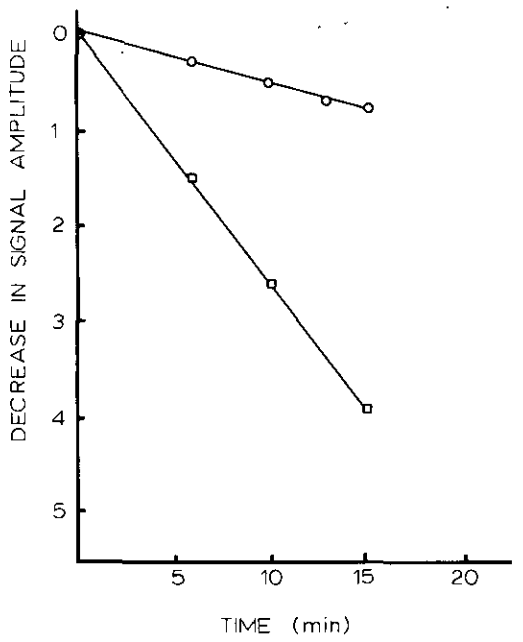


FIGURE 4. Inhibition of the reduction of free spin-labeled quat by a nitroxide reductase in guinea pig skin using DNCB as a protein modifying agent. (o) treated with DNCB; (□) without DNCB.

the spin-labeled binding site complex, then specificity could be established by quantitative exchange of the label. A kinetic study of these exchange reactions is presented in Figure 5, where fast exchange of both quats 16 and 20 occurs with greater than 90% competitive exchange. It is difficult to determine the precise concentrations of bound spin-label *in vivo* especially in 4 mm punch biopsies. Attempts to estimate concentrations reflect average spins in the EPR, and significant broadening of the nitroxide radical signal does occur upon complex formation. However, we do know that the upper limit for complex formation is approximately 10^{-7} M. The possibility that the few spin-labeled molecules which remained after exchange reactions with quats 16 and 20 may represent metabolically altered spin label capable of covalent interaction with binding sites could not be ruled out. When the tissue was homogenized in 80% trichloroacetic acid, all of the residual label was removed which indicates that only ionic complexation can be considered here. Therefore, these exchange reactions indicate that immunogenic complex formation in the epidermis can only occur through the stabilization of ion-pairs with these allergenic quats.

Discussion

The delayed hypersensitivity reactions by quats have remained controversial due to conflicting reports in the literature. Calnan (*8*) reviewed 220 patients with contact dermatitis and found that in 29 cases quats were suspected as allergens. Also, Huriez (*9*) reported that 70 of 205 cases in France were suspected due to the direct application of skin creams containing quats as preservatives.

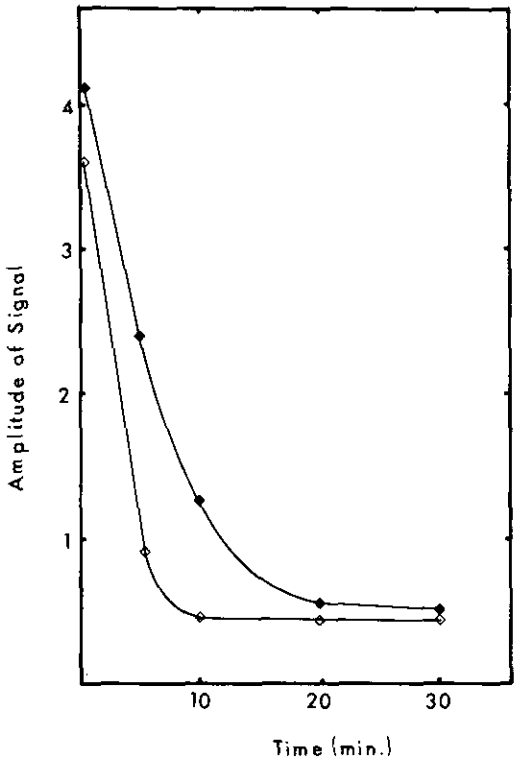


FIGURE 5. Rate of exchange of bound spin-labeled quat (intermediate allergenicity) with strongly allergenic quats 16 (♦) and 20 (◇).

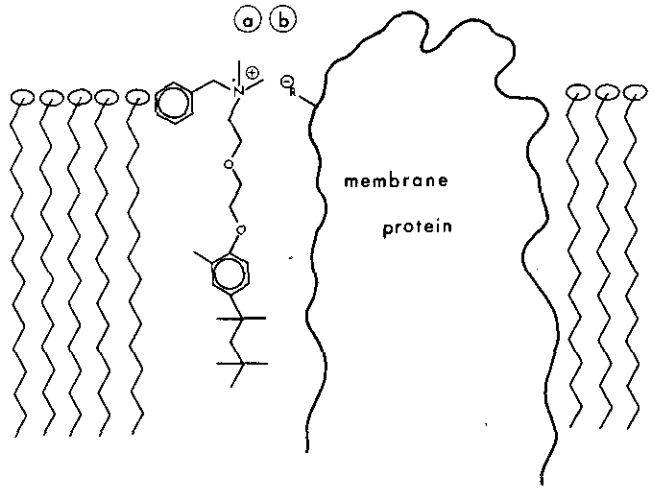


FIGURE 6. Proposed structure for the stable ion-pairs in the formation of antigens of ionic origin: (*a*) membrane complexed quat 20; (*b*) anionic binding site which forms a stable antigen with this strong allergen.

Considering the widespread use of quats in skin preparations, shampoos, medicaments, industrial cleansers, agricultural sterilizers, etc., the actual number of well established cases for allergenicity is quite small. Even so, there are a number of case histories for people who definitely show special hypersensitivity to these preservatives in shampoos, mouthwashes, plaster of paris, etc. (*10–13*).

Certain important structural features have to be met in order for these preservatives to form stable immunogenic complexes with binding sites in the skin. In this respect, quats which contain more hydrophobic groups in positions R_1 through R_4, tend to be allergenic, whereas those with hydrophilic groups (i.e., hydroxyl, amido, etc.) and which are capable of forming hydrogen bonds with water, were found to be nonallergenic. The strong allergenicity of quats 16 and 20 appears to be due to the presence of a long and linear hydrophobic group which allows direct interaction with the outer lipid layer of cell membranes to anchor the quaternary ammonium cation at the cell surface where stable complexation ensues (Fig. 6). Without this lipid-quat interaction it is unlikely that stable ion-pairing can occur because of the property of quaternary ammonium salts to dissociate to free ions in aqueous media. This lipid-quat interaction serves two purposes, one to anchor the quaternary ammonium ion in the correct orientation to creact with binding sites at the cell surface, and secondly, to add thermodynamic stability to the ion-pair, so that this complex can be recognized as a stable antigen by the immune system. Such stable complexes are not expected to be formed with cations which freely dissociate in water (e.g., quats containing hydrophilic groups).

The application of the technique of spin-labeling to this problem has been useful in several respects. Experiments are conducted *in vivo*, and therefore the technique offers a unique opportunity to examine the nature of the initial quat-binding site complex formation. The results show that stable ion-pairs are formed close to the membrane lipid surface. The kinetic study with quats of intermediate to strong allergenicity indicates that stable ion-pairs are formed with identical binding sites, especially since any loosely complexed, or free nitroxide-label, is immediately reduced by a very active nitroxide reductase in guinea pig skin. Recent experiments with human skin show us that a similar enzymatic activity is present in human epidermis (*25*). It is unlikely that this enzyme is specific for nitroxide radicals only. It seems reasonable that epidermal cells, which provide the first defense against invading foreign substances, should have evolved mechanisms for removing highly reactive free radicals before their invasion into the cytoplasm. The discovery of this enzyme is interesting in that its presence or absence in diseased skin may be of clinical significance.

Even though the technique of *in vivo* spin-labeling is able to identify the location, specificity, and dynamics of quat-binding site complex formation in guinea pig epidermis, this method does not identify which cells are involved in this rapid reaction. It is well established that epicutaneous sensitization against contact allergens is mediated by T-lymphocytes. Furthermore, these T-lymphocytes are activated by antigen which is processed and presented by Langerhans cells. Recently the importance of skin-associated lymphoid tissues (SALT)

in the presentation of contact allergens to the immune system has been fully appreciated (*26*). Antigen processing, presentation and recognition is apparently mediated in the epidermis by cells of mesenchymal origin (*27–31*). Epidermal Langerhans cells, though small in number, may contain those binding sites for the formation of immunogenic complexes with allergens (*32*). If this is the case, it would explain the importance of the dose-response characteristics for chemicals which cause contact hypersensitivity reactions. If the dose delivered to the epidermis is very small, then antigen formation with receptor sites on the small numbers of infiltrating Langerhans cells would be minimized. This would produce an insufficient concentration of antigen to imprint the T-lymphocytes to cause the immune response. However, if the concentration is too large, then toxic levels may result in cell inhibition and even death of the skin-associated lymphoid tissue so that the immune system cannot be triggered by intact antigens on viable cell surfaces. It is unlikely that membrane proteins, with their concomitant antigenic properties, will be removed specifically from membrane debris of dead cells by living cells for presentation to T-lymphocytes at the cell surfaces of those living cells. Thermodynamic arguments can be made against such direct scavenging of antigenic membrane proteins. With the quaternary ammonium compounds the importance of the formation of a sufficient concentration of stable antigen is not only highlighted, but proved by the experiments reported herein with a homologous series of quats containing an identical functional group but showing different levels of allergenicity from zero to strong. Furthermore, the spin-labeling experiments show that immunogenic complexes are formed at the cell surface, since we can find no evidence for strong immobilization which would be expected to occur if the spin-labeled antigen was taken up into the interior of cells by phagocytosis. Therefore, there is considerable information on how the immune system is triggered and controlled by allergens. What we have examined here is the initial rapid complexation reaction in the skin. This reaction takes place long before the onset of the immune response, which apparently occurs in hours rather than in milliseconds.

We wish to thank Dr. M. Dahl for his critical review of the manuscript. This research was sponsored by the Bundesministerium für Jugend, Familie und Gesundheit, the Industrieverband für Körperflege und Waschmittel and by a grant from the National Institutes of Health AM18101. This work was completed as part of an NIH postdoctoral program at the University of Minnesota. Excellent technical assistance on the electron spin resonance study was provided by S. Michurski and assistance with laboratory animals by F. Engle.

REFERENCES

1. Landsteiner, K., and Jacobs, J. L. Studies in the sensitization of animals with simple chemical compounds II. J. Exptl. Med. 64: 625–639 (1936).
2. Polak, L., Turk, J. L., and Frey, J. R. Studies on contact hypersensitivity to chromium compounds. Progr. Allergy 17: 149–226 (1973).
3. Maguire, H. C., and Chase, M. N. Studies on the sensitization of animals with simple chemical compounds XIII sensitization of guinea pigs to picric acid. J. Exptl. Med. 135: 357–375 (1972).
4. Nishioka, K. Allergic contact dermatitis. Int. J. Dermatol. 24: 1–8 (1985).
5. Herff, D. V. Sogenannte Arzneimitteldiosynkrasien. Deut. med. Wschr. 17: 1044–1051 (1937).
6. Hagermann, G., and Grubb, E. Über Invertseifen für Dermatologischen Gebrauch. Sv. Läkantidn. 39: 2265–2268 (1951).
7. Wahlberg, J. E. Two cases of hypersensitivity to quaternary ammonium compounds. Acta Derm. Venerol. 42: 230–238 (1962).
8. Calnan, C. D. Contact dermatitis from drugs. Proc. Roy. Soc. Med. 55: 39–57 (1962).
9. Huriez, C., Agache, P., Martin, P. Vandamme, G., and Menneicier, J. Un groupe d'allergenes chimiques frequement en cause: ces sels d'ammonium quaternaire. Rev. Franc. Allerg. 5: 134–152 (1965).
10. Dawber, R. P., and Calnan, C. D. Birds nest hair-matting of scalp due to shampooing. Clin. Exp. Dermatol. 1: 155–156 (1976).
11. Haidar, A. An adverse effect to a topical antiseptic (Cetrimide). Brit. J. Oral. Surgery. 15: 86–90 (1977).
12. Staniforth, P. Allergy to benzyalkonium chloride in plaster of Paris after sensitization to cetrimide. J. Bone Joint Surg. 62B: 500–502 (1980).
13. Staniforth, P., and Lovell, C. L. Contact dermatitis related to constituent of orthopaedic wool. Brit. Med. J. 283: 1285–1288 (1981).
14. Afzelius, H., and Thulin, H. Allergic reactions to benzylalkonium chloride. Contact Derm. 5: 60–72 (1979).
15. Fisher, A. A., and Stillman, M. A. Allergic contact hypersensitivity to benzalkonium chloride. Arch. Derm. 106: 169–174 (1972).
16. Gruss, M. Vergleichende Untersuchung über das Sensibilisierungsvermögen der wichtigsten quaternären Ammoniumverbindungen in kosmetischen Mitteln. Diss. Hamburg, 1983.
17. Weaver, W. E., Whaley, W. M. The synthesis of stable nitroxide radicals. J. Am. Chem. Soc. 69: 515–520 (1947).
18. Magnusson, B., and Kligman, A. M. Allergic contact dermatitis in the guinea pig. In: Identifications of Contact Allergens. Charles C. Thomas, Springfield, IL, 1970.
19. Walle, van der, H. B. Sensitizing potential of acrylic monomers in guinea pigs. Proefschrift, Leiden, 1982.
20. Ackerman, A. B. Histologic diagnosis of inflammatory skin diseases. In: A Method of Pattern Analysis. Lea and Febiger, Philadelphia, 1978.
21. Stone, T. J., Buckman, T., Nordio, P. L., McConnell, H. M. Spin-labeling of micelles. Proc. Natl. Acad. Sci. (U.S) 54: 1010–1017 (1965).
22. Melhorn, R. J., and Packer, L. Inactivation and reactivation of mitochondrial respiration by charged detergents. Biochim. Biophys. Acta 423: 382–397 (1976).
23. Piette, L. H., and Hsia, J. C. Spin-labeling of biological membranes and proteins. In: Spin Labelling, Vol. II. (L. J. Berliner, Ed.) Academic Press, New York, 1979, pp. 247–318.
24. Hsia, J. C., and Little, J. R. Alterations of antibody binding properties and active site dimensions in the primary and secondary immune response. Biochemistry 10: 3742–3749 (1971).
25. Schallreuter, K. U., and Wood, J. M. The role of thioredoxin reductase in the reduction of free radicals at the surface of the epidermis. Biochem. Biophys. Res. Comm. 136: 221–227 (1986).
26. Streilein, J. W. Circuits and signals of the skin-associated lymphoid tissue (SALT). J. Invest. Dermatol. 85: 105–135 (1985).
27. Rowden, G. The Langerhans cell. CRC Crit. Rev. Immunol. 3: 95–179 (1981).
28. Andrew, W., and Andrew, N. V. Lymphocytes in the normal epidermis of the rat and of man. Anat. Rec. 104: 217–232 (1949).
29. Katz, S. I., Tamaki, K., and Sacks, D. H. Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 282: 324–327 (1979).
30. Tschachler, E., Schuler, G., Hutterer, J., Leibel, H., Wolff, K.,

and Stingl, G. Expression of Thy-1 antigen by murine epidermal cells. J. Invest. Dermatol. 81: 282–285 (1983).
31. Bergstresser, P. R., Tigelaar, R. E., Dees, J. H., and Streilein, J. W. Thy-1 antigen bearing dendritic cells populate murine epidermis. J. Invest. Dermatol. 81: 282–288 (1983).
32. Stingl, G., Wolf-Schreiner, E. C., Pichler, W. J., et al. Epidermal Langerhans cells bear Fc and C_3 receptors. Nature 268: 245–246 (1978).