

***In vitro* and *in vivo* inactivation of reagins with mercaptans**

F. T. KISIL,* A. H. SEHON and H. Z. HOLLINGER

Department of Immunology, Faculty of Medicine, University of Manitoba, Winnipeg, and Division of Clinical Immunology, Reddy Memorial Hospital, Montreal, Canada

Summary

The ability of a series of mercaptans, i.e. 2-mercaptoethanol, l-cysteine, C-acetyl-cysteine, N-acetyl-cysteine, β - β -dimethyl-cysteine and cysteamine, to inactivate skin sensitizing antibodies in the serum of ragweed allergic individuals was studied. Following *in vitro* reductions and alkylations, 2-mercaptoethanol was shown to be the only mercaptan capable of destroying completely reaginic activity at 0.08-0.1 M. The other mercaptans, under similar conditions, were able to reduce only partially the skin-sensitizing activity. Some reaginic activity was restored if reoxidation of the reduced allergic serum was not prevented. However, it was shown that mixtures of allergic serum and mercaptan, when injected into monkey skin, did not regain skin sensitizing activity. Moreover, pre-treatment of monkey skin with mercaptan did not detectably affect the ability of the skin to fix human reagins.

C-acetyl-cysteine was used for testing its efficacy of inactivating reagins *in vivo* in monkeys; it was demonstrated that the PCA titres of a human allergic serum were thus decreased by a factor of 8 following administration of the mercaptan to monkeys.

Introduction

The common hyposensitization treatment of allergic patients consists of parenteral administration of small doses of the allergen(s). The development of blocking antibodies seems to be the major immunological response to this treatment (Sherman, 1941). In contrast to reagins, the blocking antibodies have no affinity for human skin or mucosa (Loveless, 1941) but are capable of reacting with and neutralizing the allergen (Cooke *et al.*, 1935).

Skin-sensitizing antibodies, unlike immune antibodies (IgG), are sensitive to reducing agents at low concentrations and are inactivated by treatment with 0.1 M mercaptoethanol (Leddy *et al.*, 1962; Gyenes *et al.*, 1964). If reagins could be inactivated *in vivo* by the administration of mercaptans to man, without concomitant inactivation of blocking antibody or without changing other vital functions, this

* Present address: Department of Medicine, Clinical Center, State University of New York at Buffalo, New York.

Correspondence: Dr A. H. Sehon, Department of Immunology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.

procedure might have therapeutic potential for the treatment of allergic conditions mediated by skin-sensitizing antibodies. The possibility that reagins might be destroyed or inactivated *in vivo* by the administration of mercaptans, a procedure which had been previously shown to be effective *in vitro* with mercaptoethanol (Leddy *et al.*, 1962; Gyenes *et al.*, 1964), has been explored in this study.

Materials and methods

Reducing agents

The six mercaptans (structural formulae given in Fig. 1) used to inactivate reagins were: 2-mercaptoethanol (2-ME), (Eastman Organic Chemical, Rochester, N.Y.); Mucomist (N-acetyl-cysteine) (Mead Johnson, Montreal, Quebec); Acthiol (C-acetyl-cysteine HCl) (Laboratories Joillie, France); 1-cysteine (Sigma Chemical Co., St

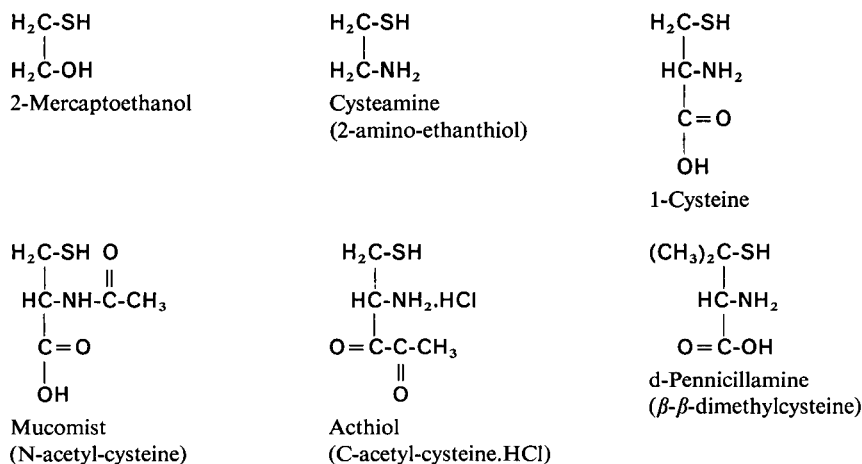


Fig. 1. Structural formulae of the mercaptans used to inactivate reagins.

Louis, Mo.); d-penicillamine (β - β -dimethylcysteine) (Merk, Sharp and Dohme, Montreal, Quebec); Cysteamine (2-amino-ethanthiol) (K & K Laboratories, Plainview, N.Y.). All mercaptans, with the exception of 2-ME and Mucomist,* were separately dried over phosphorous pentoxide. Stock solutions, containing 0.2 M of the mercaptan, were prepared in a phosphate-citrate buffer,† pH 7.1, which had been degassed and saturated with nitrogen gas immediately prior to use.

Passive cutaneous anaphylaxis (PCA)

Macaca irus monkeys were used for the evaluation by the PCA test (Ishizaka, Ishizaka & Arbesman, 1967) of the skin-sensitizing activity of sera of patients allergic to ragweed pollen. Tranquillizers or sedatives were not used. The chest and abdominal areas were closely shaved with electric clippers while the animals were restrained on their backs. To sensitize skin sites, a volume of 0.05 ml of the allergic serum samples was injected intradermally along the chest and abdominal regions. Occasionally, skin sites along the upper median area of the arms and thighs were used. Twenty-four hours following sensitization, the sites were challenged with an intravenous injection of

* Mucomist is supplied as a 20% (w/v) solution in phosphate-citrate buffer, pH 7.1.

† Disodium phosphate 0.04 M, citric acid 0.005 M, pH 7.1.

2.2–2.4 ml/kg body weight of a mixture containing equal volumes of the aqueous extract of ragweed pollen (WSR), corresponding to 20,000 PNU*/ml, and Evans blue (2%). Positive reactions as evidenced by extravasation of dye were recorded 20 min after challenge and graded from 1+ to 4+. To detect and to rule out any non-specific skin reactions, control sites were 'sensitized' with the PBS which had been used in the preparation of serum dilutions.

(A) *In vitro treatment of allergic serum with mercaptan*

To prevent the formation of a gel the allergic serum was initially diluted with an equal volume of the phosphate-citrate buffer prior to reduction. An equal volume of the mercaptan, appropriately diluted from stock solutions, was added to the diluted serum to give final concentrations ranging from 0.04 to 0.1 M. The mercaptans, L-cysteine, D-penicillamine and cysteamine, were used only at final concentrations of 0.1 M and 0.08 M.

The rate of inactivation of skin-sensitizing activity was investigated by incubating mixtures of allergic serum and mercaptan at each concentration for varying lengths of time at 37°C and the reactions were terminated by the addition of a solution of iodoacetamide (IA) (Koch-Light Laboratories, London) at alkaline pH in an amount which exceeded the original mercaptan concentration by 10%. The tubes containing allergic serum, mercaptan and IA were cooled in ice for 30 min; the individual serum samples treated with the six different mercaptans were dialysed separately against large volumes of phosphate buffered saline, pH 7.4 (PBS) at 4°C.

A 'zero time' control was arbitrarily chosen as the serum sample to which IA (in a concentration in excess of mercaptan) was added within 20 sec following the addition of the mercaptan. To test the effect of all reagents (i.e. mercaptan, buffer and IA) on the skin-sensitizing activity of allergic serum MacM, the controls illustrated in Table 3 were included. In control C-3, no IA was used; instead the treated allergic serum sample was dialysed to remove 2-ME so as to permit reoxidation of the reduced material. All control samples were separately dialysed against PBS, sterilized by filtration through Millipore membrane (0.45 μ pore size, Millipore Filter Corp., Bedford, Mass.) and assayed for skin-sensitizing activity by the PCA test in monkeys and by P-K tests in human volunteers.

(B) *In vivo studies*

(i) *Control experiments.* In view of the fact that reaginic activity was not abolished solely by treating with 2-ME (when IA was omitted) followed by dialysis (see results, page 390) the possibility existed that similar *in vivo* conditions might be encountered in which reagins would not be completely inactivated by the administration of the mercaptan without subsequent treatment with alkylating agents. To investigate this possibility aliquots of the allergic serum in two-fold dilutions were incubated with 2-ME (0.1 M final concentration) for 30 min at 37°C and volumes of 0.05 ml of the mixtures were injected intradermally into a monkey. Allergic serum in two-fold serial dilutions, similarly treated with saline, served as control.

To rule out the possibility that the mercaptans had either inactivated or altered the monkey skin for subsequent fixation of reagins, the following experiments were performed. On day 1, one group of skin sites were injected intradermally with a volume of 0.05 ml of 2-ME (0.1 M), the second group of skin sites were injected with Acthiol

* 1 PNU = 10^{-5} mg nitrogen determined by Kjeldahl analysis (McKenzie & Wallace, 1954).

(0.1 M) and the third group of skin sites with the phosphate-citrate buffer. On day 2, allergic serum MacM, or Gr, in two-fold serial dilutions, was injected into these skin sites. In addition, the allergic sera in two-fold serial dilutions were used to sensitize skin-sites which had not undergone any previous treatment. On day 3 the sites were challenged by intravenous injection of a mixture of WSR and dye.

(ii) *The inactivations of passively transferred human reagins in monkey by the in vivo administration of mercaptan.* Four monkeys weighing from 2.2 to 2.5 kg were used in the study to determine the feasibility of using mercaptans *in vivo* to inactivate skin-sensitizing activity. Each of the experiments required 4 days for their completion and was performed as outlined in Fig. 2.

Day 1 Sensitization

Skin sites in four monkeys sensitized in duplicate with 0.05 ml of allergic serum MacM, in two-fold serial dilutions.

Day 2 Treatment with mercaptan

The four monkeys received intravenous injection of 5 ml solutions of Acthiol in doses of 0.25 g, 0.75 g, 1.0 g and 1.25 g respectively.

Day 3 Sensitization (post-mercaptan treatment) control

New skin sites in each monkey sensitized with 0.05 ml of allergic serum MacM, in two-fold serial dilutions.

Day 4 Challenge

Skin sites challenged by intravenous injection of WSR and Evans blue dye solution; PCA reactions graded within 20 min.

Fig. 2. Flow sheet for the experimental procedures designed to inactivate reagins by *in vivo* administration of Acthiol and to measure the degree of inactivation.

Results

(A) *In vitro* inactivation of skin-sensitizing activity by mercaptans

The effect of the exposure of reagins for varying times and to different concentrations of 2-ME, Acthiol and Mucomist, as measured by the PCA test in *Macaca irus* monkeys, is shown in Table 1. It is evident that only 2-ME was able to inactivate completely skin-sensitizing activity within a period of 2 hr when this mercaptan was used in final concentrations greater than 0.04 M. Neither Acthiol nor Mucomist, at the concentrations and incubation times employed, could inactivate completely skin-sensitizing activity.

The results of PCA tests in another monkey (Table 2) revealed that inactivation of skin-sensitizing activity was obtained with cysteine, d-penicillamine and cysteamine when used in concentrations of 0.1 M and for periods of incubation ranging from 0 to 120 min. These mercaptans were equally effective at concentration of 0.08 M, with the exception of d-penicillamine which required a minimum incubation period of 30 min or more to completely inactivate reagins. It was surprising that PCA reactions had not been elicited with most of the 'zero time' control samples. Since skin-sensitizing antibodies may have become fixed to a lesser degree in this monkey, or since the PCA test may be less sensitive than the P-K reaction, it was deemed advisable to resort to P-K tests. Indeed the results of P-K tests with the same allergic serum samples demonstrated that while reagins were completely inactivated with 2-ME, the other mercaptans were not effective in destroying the reaginic activity completely even at the concentration of 0.1 M and at the longest incubation period of 120 min. Moreover, the inactivation of reagins with 2-ME depended on both the time of incubation and on the

Table 1. PCA reactions* observed with mercaptan treated allergic serum MacM†

Mercaptan	Final molarity	Time of incubation (min)				
		0	15	30	60	120
2-Mercaptoethanol	0.10	2	—	—	—	—
	0.08	2	1	—	—	—
	0.06	4	3	1	1	—
	0.04	2	2	1	1	1
Acthiol	0.10	2	1	1	1	1
	0.08	2	1	1	2	3
	0.06	1	1	1	2	2
	0.04	2	2	2	2	2
Mucomist	0.10	2	2	2	2	1
	0.08	2	2	2	2	2
	0.06	2	2	2	1	1
	0.04	4	4	2	2	2

* PCA reactions were graded from negative (—) to 4+.

† PCA titre of allergic serum MacM (not treated with mercaptan) was 1:256.

Table 2. PCA and P-K reactions* observed with allergic serum MacM after pre-treatment with different mercaptans

Mercaptan	Time of incubation (min)	PCA activity		P-K activity	
		0.08 M	0.1 M	0.08 M	0.1 M
2-Mercaptoethanol	0	1	—	3	3
	15	—	—	1	—
	30	—	—	1	—
	60	—	—	—	—
	120	—	—	—	—
L-Cysteine	0	—	—	3	1
	15	—	—	3	2
	30	—	—	3	1
	60	—	—	3	1
	120	—	—	2	1
d-Penicillamine	0	2	—	3	3
	15	2	—	3	3
	30	—	—	3	3
	60	—	—	3	3
	120	—	—	3	3
Cysteamine	0	—	—	3	3
	15	—	—	3	2
	30	—	—	3	2
	60	—	—	3	2
	120	—	—	3	1

* The PCA and P-K reactions were graded from negative (—) to 4+. The PCA and P-K titres of serum MacM (not treated with mercaptan) were 1:64 and 1:700, respectively.

concentration of 2-ME, i.e. at 0.1 M of 2-ME the reagins were inactivated within 15 min, but at 0.08 M of 2-ME the incubation had to be extended to 30 min for the same effect. Thus, it was concluded that the inactivation of reagins which had been apparently achieved by three of the four mercaptans, as revealed by the absence of PCA reactions, was in fact due to a decreased sensitivity of the PCA response and that only 2-ME was effective in inactivating skin-sensitizing activity completely.

In control experiments it was demonstrated that dilution of the allergic serum with buffer or saline, or incubation with IA in the absence of mercaptan, had no appreciable effect on reaginic activity (Table 3). Treatment of the allergic serum with 2-ME alone (control C-3) reduced the PCA reaction from 4+ to 2+. Although this difference in response may not be significant, the positive reactions nevertheless indicated that treatment of allergic serum MacM with 2-ME, without subsequent alkylation, was not sufficient to completely prevent reformation of reagins.

Table 3. Determination of the effect of buffer, mercaptan, and iodoacetamide on the skin-sensitizing activity of allergic serum, MacM

Control	Allergic serum (ml)	2-ME (ml)	IA (ml)	Buffer and/or saline (ml)	Reactions‡	
					PCA	P-K
C-1*	0.5	0	0	1.0	4	3
C-2†	0.5	0	0.5	0.5	3	3
C-3*	0.5	0.5	0	0.5	2	3
C-4	0	0	0	1.0	—	—

Note: All the PCA and P-K experiments were performed at the same time in the same monkey and volunteer, respectively, who were also used in another experiment, the results of which are given in Table 2.

* Control samples C-1 and C-3 were incubated at 37°C for 30 min, and then dialysed against PBS.

† Control sample C-2 was incubated at 4°C for 30 min, and then dialysed against PBS.

‡ Reactions were graded from negative (—) to 4+.

(B) *In vivo* studies

(i) *Control experiments.* The allergic serum, diluted with saline, had a PCA titre of 256. On the other hand, since no PCA reaction was detected with allergic serum after exposure to 2-ME, it must be concluded that the *in vivo* conditions were not favourable for the restoration of reaginic activity. Moreover, since the PCA titres of 256 were identical when the allergic serum was injected into sites in close proximity to or remote from skin sites which had been sensitized with allergic serum containing 2-ME, it was concluded that the mercaptan had either lost its reducing capacity shortly after intradermal injection, or did not diffuse from the injection sites to affect neighbouring sensitized sites.

The PCA titre (Table 4) of allergic serum MacM in skin sites pretreated with Acthiol was 128 as compared to a titre of 64 in normal, non-treated skin sites. With another allergic serum Gr, a PCA titre of 256 was obtained in sites preinjected with buffer alone and a titre of 512 in sites which had not been subjected to any treatment or which had been pretreated with either Acthiol or with 2-ME. In view of the inherent inaccuracies of the PCA test a two-fold difference in titre cannot be regarded as

significant. Thus, it appears that the local *in vivo* administration of mercaptans into skin sites did not detectably alter the capacity of the skin to be sensitized by reagins, nor was the ability of tissues of the treated and sensitized skin sites affected for subsequent challenge with allergen.

Table 4. Effect of local administration of mercaptans on PCA titres of two allergic sera

Allergic serum*	Pretreatment†	PCA titre‡
MacM	None	64
MacM	Acthiol	128
Gr	None	512
Gr	Buffer	256
Gr	Acthiol	512
Gr	2-ME	512
Buffer (control)	Acthiol	0
Buffer (control)	2-ME	0

* Skin sites sensitized by intradermal injection of 0.05 ml of allergic serum in two-fold serial dilutions.

† 24 hr prior to sensitization with allergic serum, 0.05 ml of either phosphate-citrate buffer, pH 7.1, or of mercaptan (0.1 M) was injected into skin sites.

‡ Reciprocal of highest dilution of allergic serum giving a positive reaction.

(ii) *The inactivation of passively transferred human reagins in monkey by the in vivo administration of mercaptan.* Two sets of PCA experiments were performed in each monkey (Table 5). The term 'treated' refers to the PCA titres observed with allergic serum MacM, when Acthiol was injected intravenously 24 hr after sensitization of the monkeys. In this experiment an attempt was made to establish if the mercaptan could inactivate the skin-fixed reagins 24 hr after passive transfer. For the sake of a 'control'

Table 5. Effect of *in vivo* administration of Acthiol on PCA titres observed with allergic serum MacM

Monkey	Dose of Acthiol (g)	PCA titre*		Decrease in titre by a factor of:
		Treated†	Control‡	
1	0.25	16	16	1
2	0.75	4	32	8
3	1.00	128	512	4
4	1.25	64	256	4

* Reciprocal of highest dilution of allergic serum giving a positive reaction.

† Treated: Acthiol was injected intravenously 24 hr after the sensitization of the monkey skin.

‡ Control: PCA titres for skin sites sensitized 24 hr after Acthiol administration.

the same monkey was used; its skin was sensitized 72 hr after initial sensitization on the assumption that any effect of the administered Acthiol would have been annulled within 24 hr after its administration.

It is apparent from the identical 'control' and 'treated' PCA titres (Table V) that Acthiol in a dose of 0.25 g did not have any detectable effect on the skin-fixed reagins. The maximum decrease in PCA titre, by a factor of 8, was observed in the monkey which had received Acthiol in a dose of 0.75 g. Increasing further the Acthiol dose to 1.0 g or 1.25 g per animal did not lead to a more significant decrease in the PCA titres. However, several hours following the intravenous administration of Acthiol in a dose of 1.25 g, the second monkey exhibited signs of damage to its central nervous system and remained in a semi-conscious state for the rest of the experiment.

Discussion

Various drugs, such as anti-histamines which neutralize vasoactive agents liberated during the reaction of reagins and allergens, provide an immediate relief of the distresses experienced by an allergic individual, but this effect is temporary and no changes occur in the immunological parameters in hypersensitivity reactions. On the other hand, long-term treatments are based on a course of immunotherapy designed to alter the relative concentration of the two classes of antibodies (reagins and blocking antibodies) which participate in allergic reactions. While the mechanisms whereby improvement is brought about have not been elucidated, the relief experienced by allergic patients receiving hyposensitization treatment may be attributed, in part, to the formation of blocking antibodies which neutralize the allergens and thus prevent these from reaching and reacting with reaginic antibodies fixed to the cells of target organs and, in part, to a decrease in the level of reagins (Connell & Sherman, 1969; Tse, Wicher & Arbesman, 1970). For the latter observation it has been suggested that reagins are either neutralized or that a partial tolerance to the specific allergen may be induced by hyposensitization treatment (Malley & Perelman, 1970). However, hyposensitization treatments always carry the inherent danger of anaphylactic reactions. Moreover, immunotherapy with crude extract (Richter *et al.*, 1958) or with low molecular weight preparations of allergens (Malley & Perelman, 1970) have led to complications due to the formation of reaginic antibodies of different specificities from those before treatment. In the present investigation the possibility that reagins might be inactivated *in vivo* was explored.

The selection of the mercaptans used in this study was based partly on the similarity of their chemical structure to 2-ME, which had been shown to inactivate reaginic activity, and partly on their low levels of toxicity. Cysteamine which forms a part of coenzyme A is most similar to 2-ME, the hydroxyl group being replaced by an amino group. Moreover, it was claimed to be capable of inhibiting both the tuberculin reaction in sensitized humans and the passive Arthus reaction in the skin of rabbits (Lecomte & Borensztajn, 1953). The four remaining mercaptans were cysteine, a natural amino acid, and its three derivatives. Thus, Acthiol is the C-acetyl derivative of cysteine; Mucomist (i.e. N-acetyl cysteine) had been used in the treatment of chronic bronchitis in man (Webb, 1962) in the belief that its mucolytic action was due to breaking of the disulphide bonds of mucus; d-penicillamine was chosen, because it had been shown to reduce the concentration of the serum macroglobulin paraprotein component (IgM) when administered to patients with Waldenström's macroglobulinaemia (Bloch, Prasad & Anastasi, 1958).

The results of the *in vitro* control experiments clearly established that reagins were not demonstrably affected by such procedures as dilution of allergic serum with the phosphate-citrate buffer, incubation with mercaptans at 37°C (for a minimum 'zero' time), or dialysis against PBS. A variability of PCA titres obtained in different monkeys emphasized the importance of including the identical control (i.e. the same allergic serum in two-fold serial dilutions) throughout the PCA tests. In addition, in all *in vitro* experiments, the capacity of different mercaptans to inactivate reagins was always compared to that obtained with 2-ME. The differences between PCA and P-K titres of allergic sera can be accounted for by the lower sensitivity of the PCA reaction in monkey skin (Rose *et al.*, 1964; Patterson *et al.*, 1966) and the variation of PCA reactions observed in monkeys appear to be similar to variations of P-K reactions observed in man.

It was unequivocally established that 2-ME was the most effective mercaptan for the inactivation of reagins by the *in vitro* treatment of allergic serum. The other five mercaptans, in comparable concentrations, were capable of only partially reducing reaginic activity. Reaginic activity could be recovered when reduced allergic serum was allowed to reoxidize indicating that both skin-fixing and antibody combining capacity could be recovered. On the other hand, when the reduced preparation was treated with iodoacetamide (a procedure which by itself does not inactivate intact reagins), reaginic activity was not restored.

Since it was conclusively demonstrated that the two mercaptans, 2-ME and Acthiol, did not by themselves inactivate or alter the skin for fixation of passively transferred reagins, the failure to elicit positive PCA reactions in skin sites sensitized with a mixture of allergic serum and 2-ME indicates that either the skin-fixing portion of reagent molecules or the antibody combining sites, or both, were not reformed *in vivo* to an active configuration following the elimination or inactivation of mercaptan from the skin sites.

In another study (Ishizaka & Ishizaka, 1968) it has been revealed that following reduction and alkylation, the antibody activity was reduced by one-third and a five-fold increase in the concentration of the reduced and alkylated material was required to block passive sensitization in comparison with the untreated reaginic serum fraction. It must therefore be concluded that both the skin-fixing capacity and antibody combining sites of reagins were impaired by reduction when followed by alkylation.

In the *in vivo* studies it was anticipated that inactivation and elimination of the injected mercaptan would occur in addition to its dilution and escape into extravascular spaces. Thus, only a small portion of the doses of Acthiol with reducing capability could conceivably reach the sensitized skin sites. The results of the *in vitro* experiments had already indicated that Acthiol at 0.1 M was not sufficient to inactivate completely reagins of the six-fold diluted allergic serum. Nevertheless, an eight-fold reduction in PCA titre following *in vivo* treatment with mercaptan revealed that some skin-sensitizing antibodies had indeed been inactivated by the intravenously administered Acthiol.

It is possible that this decrease in PCA titres may be accounted for by the elimination of reagins from the passively sensitized monkey skin sites by processes of catabolism during the 48 hr between the two sensitizations. However, this seems unlikely in view of the fact that in the animal treated with Acthiol in a dose of 0.25 g identical PCA reactions were observed in skin sites sensitized 2 days apart with the allergic

serum. It is worth noting that in another study designed to examine the effect of tranquilizers on the skin reactivity of monkeys sensitized locally with allergic sera, it was found that the PCA titre of a ragweed allergic serum was reduced from 125 to 25 following the intramuscular administration of acepromazine; a phenothiazine with a sulphydryl group (Feinberg, Feinberg & Lee, 1969).

The cumulative results of the *in vitro* and *in vivo* experiments indicate that excessively large amounts of mercaptan, in doses toxic to the animals, would have to be administered to inactivate completely all reaginic activity. It must therefore be concluded that no satisfactory mercaptan was found which could be used safely to inactivate completely skin-fixed reagins and which could be justifiably administered to man for the same purpose.

References

- BLOCH, H.S., PRASAD, A. & ANASTASI, A. (1958) Serum protein changes in Waldenström's macroglobulinemia during penicillamine administration. *Journal of Laboratory and Clinical Medicine*, **52**, 793.
- CONNELL, J.T. & SHERMAN, W.B. (1969) Changes in skin-sensitizing antibody titer after injections of aqueous pollen extract. *Journal of Allergy*, **43**, 22.
- COOKE, R.A., BARNARD, J.H., BEBALD, S. & STULL, A. (1935) Serological evidence of immunity with coexisting sensitization in type of human allergy (hay-fever). *Journal of Experimental Medicine*, **62**, 733.
- FEINBERG, A.R., FEINBERG, S.M. & LEE, F. (1969) Hypersensitivity responses in monkeys. 3. The influence of various tranquilizers and other drugs on immediate skin reaction. *Journal of Allergy*, **43**, 81.
- GYENES, L., SEHON, A.H., FREEDMAN, S.O. & OVARY, Z. (1964) The properties of fragments of skin-sensitizing and blocking antibodies as revealed by the Prausnitz-Küstner, passive cutaneous anaphylaxis and hemagglutination reactions. *International Archives of Allergy and Applied Immunology*, **24**, 106.
- ISHIZAKA, K. & ISHIZAKA, T. (1968) Human reaginic antibodies and immunoglobulin E. *Journal of Allergy*, **42**, 330.
- ISHIZAKA, K., ISHIZAKA, T. & ARBESMAN, C.E. (1967) Induction of passive cutaneous anaphylaxis in monkeys by human gamma-E antibody. *Journal of Allergy*, **39**, 254.
- LECOMTE, J. & BORENSZTAJN, C. (1953) Action of phenergan on the intradermal tuberculin reaction. *Compte Rendu des Séances de la Société de Biologie*, **147**, 359.
- LEDDY, J.P., FIREMAN, G.L., LUZ, A. & TODD, R.H. (1962) Inactivation of the skin-sensitizing antibodies of human allergy by thiols. *Proceedings of the Society for Experimental Biology and Medicine*, **111**, 7.
- LOVELESS, M.H. (1941) Immunological studies of pollinosis; passive sensitization of man through transfusion. *Journal of Immunology*, **41**, 15.
- MALLEY, A. & PERELMAN, F. (1970) Timothy pollen fractions in treatment of hayfever. I. Clinical and immunological response to small and higher molecular weight fractions. *Journal of Allergy*, **45**, 14.
- MCKENZIE, H.A. & WALLACE, H.S. (1954) Kjeldahl determination of nitrogen: a critical study of digestion conditions—temperature, catalyst and oxidizing agent. *Australian Journal of Chemistry*, **7**, 55.
- PATTERSON, R., FINK, J.N., WENNERMARK, J., BAUM, J., PRUZANSKY, J.J. & NISHIMURA, E.I. (1966) The biologic consequences of the immediate type hypersensitivity transferred from man to monkey. *Journal of Allergy*, **37**, 295.
- RICHTER, M., SEHON, A.H., GORDON, J., GRÉGOIRE, C. & ROSE, B. (1958) Demonstration of reagin of a new specificity in sera of treated ragweed-sensitive persons. *Journal of Allergy*, **29**, 287.
- ROSE, N.R., KENT, J.H., REISMAN, R.E. & ARBESMAN, C.E. (1964) Demonstration of human reagin in the monkey. I. Passive sensitization of monkey skin with sera of untreated atopic patients. *Journal of Allergy*, **35**, 520.
- SHERMAN, W.B. (1941) Changes in serological reactions and tissue-sensitivity in hay-fever patients during early months of treatment. *Journal of Immunology*, **40**, 289.

- TSE, K.S., WICHER, K. & ARBESMAN, C.E. (1970) IgE antibodies in nasal secretions of ragweed-allergic subjects. *Journal of Allergy*, **46**, 6.
- WEBB, R.W. (1962) Clinical evaluation of a new mucolytic agent, acetyl-cysteine. *Journal of Thoracic and Cardiovascular Surgery*, **44**, 330.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.