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MODE OF ACTION OF DISODIUM CROMOGLYCATE, STUDIES ON IMMEDIATE TYPE HYPERSENSITIVITY REACTIONS USING 'DOUBLE SENSITIZATION' WITH TWO ANTIGENICALLY DISTINCT RAT REAGINS

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SUMMARY

The mode of action of disodium cromoglycate has been investigated to determine at what stage in immediate type hypersensitivity reactions the compound is effective. *In vitro* studies using rat subcutaneous connective tissue sensitized with rat reagin revealed that the compound inhibited the allergic release of histamine if present during antigen challenge. The presence of the compound during sensitization had no effect on antigen-induced release of histamine provided the compound was removed prior to antigen challenge. Tissues which had undergone a primary antigen challenge in the presence of disodium cromoglycate did not release histamine when the compound was removed and the tissues rechallenged. These findings indicated that antigen/antibody interaction occurred in the presence of the compound resulting in desensitization to a subsequent antigen challenge. To corroborate the evidence of the *in vitro* studies *in vivo* passive cutaneous anaphylactic reactions (PCA) were undertaken using tissue sites sensitized with two reaginic antibodies which permitted a sequence of antigen challenges. Results from these *in vivo* reactions demonstrated that it was possible to desensitize tissue, without the release of the mediators of anaphylaxis, by an antigen challenge and disodium cromoglycate treatment. In these sites sensitized with two antibodies the immunological reactivity was maintained following a primary antigen challenge and disodium cromoglycate treatment, as a subsequent challenge with the dissimilar antigen produced a good PCA reaction. It would appear that disodium cromoglycate acts either directly or indirectly at a stage following antigen/antibody reaction but prior to the release of the mediators of anaphylaxis.

INTRODUCTION

Disodium cromoglycate (DSCG) must now be considered to have a place in the prevention or prophylaxis of acute attacks in patients with allergic bronchial asthma (Pepys, 1969)

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Nevertheless, opinion is divided as to the basis for selection of patients for DSCG therapy since encouraging results have also been observed in some asthmatics in whom the attacks of asthma may be triggered by apparently non-antigenic stimuli, i.e. the individual attack may not be immunologically triggered (Reed, 1969). Hence, although a full understanding of the drug's mode of action is not a necessary prerequisite for successful therapy, the knowledge of how DSCG works could be of practical assistance in defining more precisely the indications for its use in the whole spectrum of airway disease.

Initially the beneficial effects of DSCG were revealed in asthma induced by antigen provocation tests to man (Altounyan, 1967), and therefore we began our investigations on the possible mechanism of action of the drug with animal models of immediate type hypersensitivity. Detailed studies by Goose & Blair (1969) on passive cutaneous anaphylaxis (PCA) in the rat sensitized with homologous reaginic antibody revealed that inhibition occurred only when DSCG was present at the time of antigen/antibody union. However, the homologous PCA reaction in guinea pigs induced by a homocytotropic but non-reaginic 7Sγ_1 antibody was not inhibited by DSCG (Cox, 1967; Assem & Mongar, 1970). From the available evidence we concluded that although DSCG was not an antagonist of histamine or SRS-A, it nevertheless selectively inhibited certain immunological reactions involving mast cells and reaginic antibodies by preventing the release of the pharmacological mediators.

Further work has made this simple explanation incomplete. Pepys and his colleagues (1968) have demonstrated that in some patients with bird fanciers' disease, in whom there was no immediate asthmatic reaction, the late asthmatic response to inhaled antigen was inhibited by a single prechallenge dose of DSCG. Sera from these patients contained precipitating, but not reaginic, antibodies to avian antigens. Furthermore, Morse, Austen & Bloch (1969) found that DSCG not only suppressed histamine release from rat peritoneal mast cells sensitized with rat reagin, but also suppressed histamine release from mast cells sensitized with a heat stable IgGa antibody; in our laboratories we have observed inhibition of histamine release and the 4 hr PCA in the rat, mediated by a heat stable anti-DNP 7Sγ_2 antibody fraction (Orr, Gwilliam & Cox, 1970). From these studies it is apparent that DSCG is not specific for reagins as was originally supposed, but that it may well affect other systems involving sensitization of mast cells (Lopez & Bloch, 1969). Although it cannot be assumed *a priori* that the action of the drug is identical in all systems, it is appropriate to consider in the first instance those involving reagins. Consequently, we have undertaken experiments *in vivo* and *in vitro* using rat tissue sensitized with rat reagin produced either in response to infection with *N. brasiliensis*, or in response to sensitization with egg albumin/*B. pertussis*. *In vitro* studies were used primarily as a more flexible system in which antigen, or DSCG, or both, could be added to the tissue and later removed by washing procedures which are not possible in the *in vivo* system. These studies were designed to locate the stage at which DSCG was effectively inhibiting the release of histamine from mast cells sensitized with reaginlike antibodies.

Three areas were selected for this study; the sensitization procedure, the interaction of antigen and antibody and the release of the pharmacological mediators of anaphylaxis.

Whilst certain information was obtained from the *in vitro* system, it was also felt desirable to carry out *in vivo* work to establish the immunological reactivity of the system. In particular, a technique was developed which involved double sensitization of a tissue site with two antigenically distinct rat reagins, permitting the initial antigen challenge and treatment

to be followed by a second antigen challenge. This technique was incorporated into a series of PCA reactions in the rat in such a manner that it was possible to use one antibody/antigen reaction to demonstrate inhibition by DSCG and the second antibody/antigen reaction to serve as an index of any changes in the immunological reactivity of the tissue (i.e. mast cells) induced by the initial challenge.

MATERIALS AND METHODS

Female Sprague-Dawley rats were used throughout.

Anti-sera and antigens

Nippostrongylus brasiliensis antisera and antigen were prepared and standardized using the methods described by Goose & Blair (1969).

Rat anti-egg albumin reaginic antisera were prepared using the method described by Mota (1964).

All sera were tested for reaginic activity by heat lability and long term tissue fixation in homologous passive cutaneous anaphylaxis (PCA).

In vitro technique

Pieces of subcutaneous connective tissue were removed by dissection from rats immediately after pithing and placed in Tyrode's solution. For sensitization, pieces of tissue 10–20 mg fresh wt. were placed individually in bottles containing either 2 ml of *N. brasiliensis* reaginic antisera diluted 1/4 with Tyrode or 2 ml of normal rat serum diluted 1/4 with Tyrode (controls). Sensitization was carried out by overnight incubation at 20°C followed by 1 hr incubation at 37°C in a shaking water bath (Sheard, Killingback & Blair, 1967). For antigen challenge, tissues were transferred to bottles containing either 1·8 ml fresh Tyrode (antigen challenge groups) or 2 ml fresh Tyrode (unchallenged control groups) at 37°C. After incubation for 15 min those bottles containing 1·8 ml Tyrode were challenged with 0·2 ml *N. brasiliensis* antigen to give a final antigen concentration in the bottle of 1 mg/ml total worm protein. The bottles containing the test and control tissues were incubated for a further 15 min prior to the transfer of each tissue to 2 ml fresh Tyrode. The two sets of bottles, those containing the histamine released by the tissues and those containing the tissues themselves, were boiled for 5 min to release residual histamine from the tissues. After boiling the bottles were cooled and either assayed immediately for histamine or within 24 hr of the experimental procedure after storage at −20°C.

Histamine assays were carried out using isolated guinea-pig ileum (Sheard & Blair, 1970).

In vivo double sensitization techniques for PCA activity

Anti-*N. brasiliensis* and anti-egg albumin reaginic sera were diluted with physiological saline so that 0·1 ml mixture of the two antibodies injected i.d. was capable of eliciting a PCA reaction of approximately 25 mm on intravenous challenge 24 hr later, with either antigen and 0·25 ml of 1·25% solution of Evans Blue.

RESULTS

In vitro studies

The results shown in Table 1 indicate that successful sensitization of rat subcutaneous connective tissue with rat anti-*N. brasiliensis* reagin was obtained using the *in vitro* system. Only those tissues sensitized with antibody and challenged with antigen released histamine, whereas tissues sensitized and not challenged, or unsensitized tissue that was challenged, showed little or no histamine release.

TABLE 1. *In vitro* sensitization of rat subcutaneous connective tissue with anti-*N. brasiliensis* rat reaginic serum

Treatment of tissues	Histamine release (mean of five tissues) (%)
Unsensitized, challenged	2·3 (1·8—2·6)*
Sensitized, not challenged	4·8 (2·8—6·8)
Sensitized, challenged	24·7 (12·1—36·1)

* Figures in parenthesis indicate range.

The inhibitory effect of DSCG on this system was estimated using a concentration known to inhibit antigen induced histamine release from human lung *in vitro* (Sheard & Blair, 1970). DSCG, 10 μg/ml, added to Tyrode prior to antigen challenge was observed to inhibit the antigen induced release of histamine, Table 2A.

Using DSCG at this defined inhibitory level, the effect of the compound on the sensitization of tissues was investigated. Pieces of subcutaneous connective tissue were incubated in antibody/Tyrode mixture or in antibody/Tyrode mixture containing 10 μg/ml DSCG. After the incubation procedure the tissues were washed twice in Tyrode for 5 min at 37°C before being placed in 1·8 ml or 2 ml Tyrode prior to antigen challenge.

TABLE 2. Effect of DSCG on antigen induced release of histamine from tissues sensitized *in vitro*

A. DSCG present during antigen challenge	
Treatment of tissues	Histamine release (mean of five tissues) (%)
Sensitized unchallenged	(No detectable release)
Sensitized, challenged	31·0 (23·4—50)*
Sensitized, challenged in presence of 10 μg/ml DSCG	14·8 (4·4—21·2)
B. DSCG present during *in vitro* sensitization only	
Sensitized in presence of DSCG 10 μg/ml, washed but not challenged with antigen.	2·3 (1·5—3·5)*
Sensitized in presence of DSCG, 10 μg/ml, washed and challenged with antigen.	25·2 (11·3—51·4)
Sensitized, washed and challenged with antigen.	25·5 (18·9—38·8)

* Figures in parenthesis indicate range.

The results in Table 2B show that the presence of 10 μg/ml DSCG during sensitization did not affect subsequent histamine release induced by antigen challenge if the tissues were washed to remove the compound prior to challenge. However, when DSCG 10 μg/ml was present during antigen challenge, Table 2A, the release of histamine was inhibited.

These findings suggested that DSCG might interfere either with antigen/antibody interaction or act at some stage following antigen/antibody interaction. To determine whether the compound inhibited the antigen/antibody interaction pieces of sensitized tissue were challenged with antigen in the presence of 10 μg/ml DSCG, incubated for 15 min at 37°C in Tyrode and washed twice in fresh Tyrode at 37°C to remove the inhibitory dose of DSCG. To test whether the tissues were still sensitized the washed tissues were subjected to a further 15 min incubation in Tyrode/antigen mixture and controls were re-incubated for 15 min in Tyrode only. Control sensitized tissues subjected to these incubation and washing procedures showed a normal allergic histamine release when incubated for a further 15 min in Tyrode/antigen. The results shown in Table 3 indicate that initial challenge without DSCG gave a 37·0% histamine release; in the presence of 10 μg/ml DSCG the release was substantially inhibited (10·2% and 13·2% histamine release). Washing off the DSCG and rechallenging with antigen produced a histamine release of 3·6%, i.e. below the control figure of 8·8% indicating that there was no significant release of histamine.

These results show that passively sensitized tissue subjected to an antigen challenge in the presence of DSCG resulting in a severely inhibited histamine release, is unable to react to a further antigen challenge in the absence of the compound. This suggests that the initial antigen challenge in the presence of DSCG desensitized the tissues.

TABLE 3. Effect of repeat antigen challenge using *N. brasiliensis* antigen on sensitized tissue following primary challenge

Treatment of tissues	Histamine release (mean of five tissues) (%)
Unsensitized, challenged	8·8 (2·5—15·5)*
Sensitized, challenged	37·0 (30·3—51·3)
Sensitized, challenged in presence of 10 μg/ml DSCG first release	13·2 (5·9—30·0) 10·2 (5·8—13·5)
Washed, *no rechallenge* second release	3·0 (1·5—4·6)
Washed and *rechallenged* with antigen, second release	3·6 (1·1—7·8)

* Figures in parenthesis indicate range.

In vivo PCA studies using two antibodies for sensitization

Using two antigenically distinct rat reagins a series of PCA reactions were carried out to determine whether it was possible to sensitize the same skin sites with two rat reagins to two antigens (Fig. 1). Anti-*N. brasiliensis* and anti-egg albumin sera were used at dilutions capable of inducing approximately 25 mm diameter PCA reactions on antigen challenge 24 hr after sensitization. The results of individual antibody sensitization are shown in Table 4 where anti-*N. brasiliensis* reagin on *N. brasiliensis* antigen challenge had a PCA reaction size of 23·6 mm and the anti-egg albumin reagin sites a 27·8 mm reaction on egg albumin challenge. Challenging sites sensitized only to *N. brasiliensis* antigen with egg

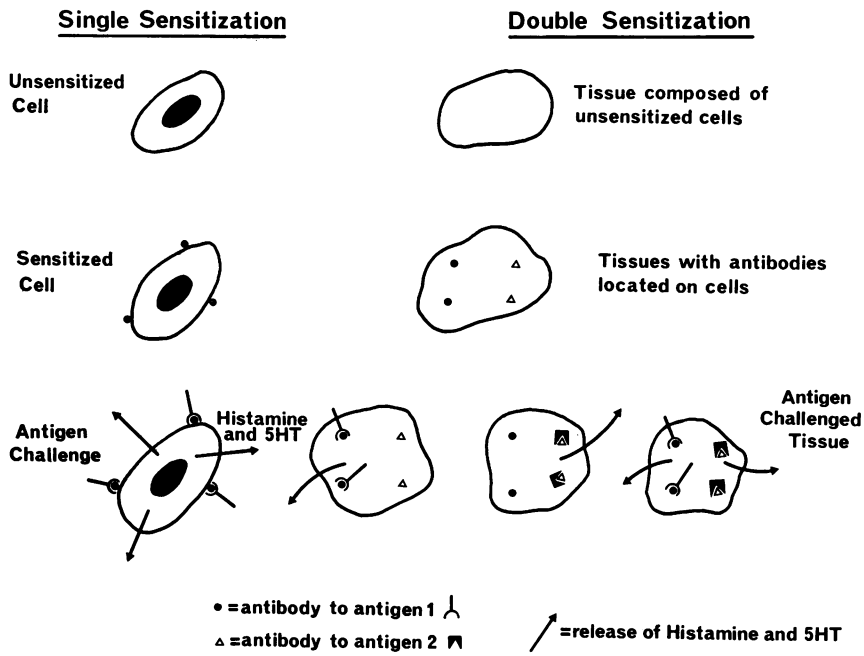

FIG. 1. Schematic diagram of single sensitization and antigen challenge compared with double sensitization in rat tissue, indicating that either or both antigens are capable of mediating the release of histamine and 5HT from sensitized tissue. See Table 4.

albumin produced no PCA reaction, as did challenging sites sensitized only to egg albumin with *N. brasiliensis* antigen, see Table 4. For double sensitization a single intradermal injection was used for both anti-*N. brasiliensis* and anti-egg albumin reagin, the final dilution of each antibody in this 0·1 ml injection being the same as that used for single antibody sensitization. The results shown in Table 4 indicate that sensitization with anti-*N. brasiliensis* and anti-egg albumin reaginic sera produced, on respective antigen challenge, approximately equal PCA reaction sizes of 27·9 mm and 24·6 mm respectively. Simultaneous challenge with both antigens, i.e. *N. brasiliensis* antigen and egg albumin, produced a larger reaction of 38·8 mm.

To utilize this double sensitization technique for testing the reactivity of sensitized sites *in vivo*, a series of experiments were designed such that a double sensitized site was challenged twice, in sequence, either with the same antigen or dissimilar antigens. These repeated challenges using either the same or dissimilar antigens were performed at various time intervals after the initial challenge, see Table 5. The results indicate that following double sensitization with anti-*N. brasiliensis* reagin and anti-egg albumin reagin, a primary challenge with *N. brasiliensis* antigen prevents a PCA reaction developing on repeat challenge with the same antigen. Similarly double sensitization followed by initial challenge with *N. brasiliensis* prevents a PCA reaction developing on repeat challenge with egg albumin up to 48 hr after the first challenge. Similar results were obtained using egg albumin for the initial challenge and either egg albumin or *N. brasiliensis* antigen for the repeat challenge. It would appear therefore that using this double sensitization technique and time schedule an initial challenge

TABLE 4. 24 hr PCA reaction in the rat induced by two antigenically distinct antibodies using separate and concurrent sensitization and challenge procedures

Sensitizing antibody	Antigen used for challenge 24 hr after sensitization	PCA reaction size (mean of five results) (mm)
Nb	Nbag	23·6
Nb + EA	Nbag	27·9
Nb	EAag	0
EA	EAag	27·8
Nb + EA	EAag	24·6
EA	Nbag	0
Nb + EA	EAag + Nbag	38·8

Nb = Anti-*N. brasiliensis* reaginic serum.
Nbag = *N. brasiliensis* antigen.
EA = Anti-egg albumin reaginic serum.
EAag = Egg albumin antigen.

with either antigen precludes the development of a PCA reaction on repeat challenge with either antigen.

To demonstrate that the inhibition of the PCA reaction following sequential administration of dissimilar antigens was not due to non-specific antibody blocking at the initial challenge, rats were singly sensitized and challenged at 24 hr with the wrong antigen, i.e. anti-*N. brasiliensis* sensitized rats were challenged with egg albumin and anti-egg albumin sensitized rats were challenged with *N. brasiliensis* antigen. 4 hr after this non-specific challenge the animals were rechallenged with the appropriate antigen. The results in

TABLE 5. Effect of sequential antigen challenge using the same or dissimilar antigens on sites sensitized with two antibodies

Sensitization	First challenge	PCA reaction first challenge	Second challenge at either 30 min, 1 hr or 6 hr	PCA reaction size of second challenge (mean of five results) at each time (mm)		
				30 min	1 hr	6 hr
Group 1 Nb+EA	Nbag	(+)	Nbag	0	0	0
Group 2 Nb+EA	Nbag	(+)	EAag	0	0	0
Group 3 Nb+EA	EAag	(+)	EAag	0	0	0
Group 4 Nb+EA	EAag	(+)	Nbag	0	0	0

Nb = Anti-*N. brasiliensis* reaginic serum. Nbag = *N. brasiliensis* antigen.
EA = Anti-egg albumin reaginic serum. EAag = Egg albumin antigen.
(+) — Denotes positive PCA reaction in controls.
Groups 2 and 4 were also given second challenge 48 hr after first challenge—no PCA reactions were produced.

Table 6 show that treatment with the wrong antigen at 24 hr had no effect on the PCA reaction induced with the correct antigen 4 hr later, as compared with controls challenged only with the appropriate antigen at 28 hr.

Using the repeat challenge system with either the same or dissimilar antigen, the effect of DSCG on the initial reaction was investigated. DSCG at 10 mg/kg completely inhibited the PCA reaction induced by either egg albumin or *N. brasiliensis* antigen challenge on doubly sensitized sites, see Table 7A. Rechallenging the system 30 min after an initial challenge with *N. brasiliensis* antigen in the presence of DSCG with a second challenge of *N. brasiliensis* antigen failed to elicit a PCA reaction. However, when the second challenge involved a

TABLE 6. Effect of using two distinct sequential antigen challenges on PCA sites sensitized to one antibody

Sensitization	Antigen used for challenge 24 hr after sensitization	PCA reaction size (mean of five results) (mm)	Treatment at 28 hr	PCA reaction size (mean of five results) (mm)
Nb	None	—	Nbag	27·8
Nb	EAag Challenge	0	—	—
Nb	EAag Challenge	0	Nbag	30·5
EA	None	—	EAag	27·2
EA	Nbag Challenge	0	—	—
EA	Nbag Challenge	0	EAag	26·5

Nb = Anti-*N. brasiliensis* reaginic sera. EA = Anti-egg albumin reaginic sera.
Nbag = *N. brasiliensis* antigen. EAag = Egg albumin antigen challenge.

TABLE 7. Effect of second antigen challenge on site previously challenged with antigen and 10 mg/kg DSCG

A. Effect of DSCG on primary challenges

Sensitizing antibody	Challenge and treatment	PCA recreation size (mean of five results) (mm)
Nb+EA	Nbag	23·4
Nb+EA	Nbag+10 mg/kg DSCG	0
Nb+EA	EAag	25·6
Nb+EA	EAag+10 mg/kg DSCG	0

B. Effect of repeat antigen challenge using similar or dissimilar antigen following primary challenge in presence of 10 mg/kg DSCG

Sensitizing antibody	First challenge and treatment	Second challenge after further 30 min	PCA reaction size (mean of five results) (mm)
Nb+EA	Nbag+10 mg/kg DSCG	Nbag	0
Nb+EA	Nbag+10 mg/kg DSCG	EAag	17·1
Nb+EA	EAag+10 mg/kg DSCG	EAag	0
Nb+EA	EAag+10 mg/kg DSCG	NBag	25

NB = Anti-*N. brasiliensis* reaginic serum. Nbag = *N. brasiliensis* antigen.
EA = Anti-egg albumin reaginic serum. EAag = Egg albumin antigen.

dissimilar antigen, i.e. with *N. brasiliensis* in the presence of DSCG 10 mg/kg followed 30 min later by challenge with egg albumin, or egg albumin and DSCG 10 mg/kg followed 30 min later by *N. brasiliensis*, good PCA reactions were produced on the second challenge.

These results indicate that an initial challenge with either *N. brasiliensis* or egg albumin in the presence of 10 mg/kg DSCG desensitized the tissue, as subsequent challenge with the same antigen produced no PCA reaction. However, this is not due to the inability of the tissue to react, as an initial challenge with either *N. brasiliensis* or egg albumin in the presence of DSCG followed by a subsequent challenge with a dissimilar antigen produces a PCA reaction. These results show that the initial challenge in the presence of DSCG desensitizes the tissue site to a further challenge with the same antigen, suggesting that antibody/antigen combination takes place in the presence of the compound.

DISCUSSION

The present results reflect our attempts to determine at which stage DSCG is effective in inhibiting immediate type hypersensitivity reactions using rat mast cells sensitized with *N. brasiliensis* anti-serum as an animal model. Sensitization of rat subcutaneous connective tissue with rat reagin was achieved in the *in vitro* studies which enabled the effect of the presence of the compound on the sensitization stage to be investigated. The presence of the compound during sensitization showed little or no effect on antigen induced histamine release, Table 2B, whereas the presence of the compound during antigen challenge clearly showed inhibition of this release, Table 2A. These results confirm and extend the initial findings of Goose & Blair (1969), Morse *et al.* (1969), Sheard & Blair (1970) and Assem & Mongar (1970), who demonstrated that DSCG present during antigen challenge effectively inhibited histamine release from tissue sensitized with reaginic antibody.

It is generally accepted that the allergic release of histamine from sensitized mast cells results from the interaction of antigen and antibody (Brocklehurst, 1968). The fact that DSCG inhibited the reaction only when present at antigen challenge could be interpreted as evidence for interference with the antigen/antibody combination or an immediately subsequent stage. Since DSCG is effective in inhibiting immediate type reactions induced by a number of antigen/antibody interactions (Cox, 1967) it was considered unlikely that the compound would interfere with such a variety of specific reactions involving different antigen/antibody combinations. Indeed the results of the *in vitro* work show that it is possible to desensitize tissue with antigen in the presence of DSCG, Table 3, and suggest that there was no active antibody available for antigen interaction during the second challenge, confirming that during the initial challenge, antigen/antibody combination had taken place in the presence of the drug, even though it did not lead to the release of histamine.

To eliminate the possibility that this inhibition by DSCG was due to the inability of sensitized mast cells to react to a subsequent challenge *in vitro*, a technique of double sensitization using two antigen/antibody systems was developed for *in vivo* use. Rat skin was sensitized with two reaginic antibodies, anti-*N. brasiliensis* and anti-egg albumin and DSCG was found to be equally effective against the PCA reaction induced by both antigen/antibody systems, Table 4. Of particular interest was the finding that complete inhibition of the PCA reaction was obtained when a subsequent challenge using the same antigen followed 30 min after a first challenge in the presence of DSCG, Table 7B. The mast cells were still capable of reacting, as the subsequent challenge using the dissimilar antigen produced a normal PCA

reaction when DSCG was present during the primary challenge. It was also noted that an initial challenge without concomitant DSCG treatment inhibited the production of a PCA reaction on subsequent challenge with either the same or dissimilar antigens, Table 5.

These *in vivo* findings lead to two important conclusions. Firstly they endorse the *in vitro* results which suggested that antigen/antibody combination occurs in the presence of the inhibitor and, secondly, they suggest that the mode of action of DSCG is concerned either directly or indirectly, with a stage following antigen/antibody combination and before the release of chemical mediators. Furthermore these results imply that the drug has no irreversible effect on the integrity of the sensitized mast cell.

Although the detailed sequence of events in antigen-induced release of histamine from sensitized rat mast cells has not been defined, the hypothesis proposed by Stanworth (1969) for human cell-bound reagin and allergen (antigen) merits careful consideration and may well correspond to conditions in certain other species. It is believed that passive sensitization involves attachment of IgE antibodies to the cell via their Fc regions, thereby leaving their Fab regions free for subsequent combination with antigen, as shown diagrammatically in Fig. 2.

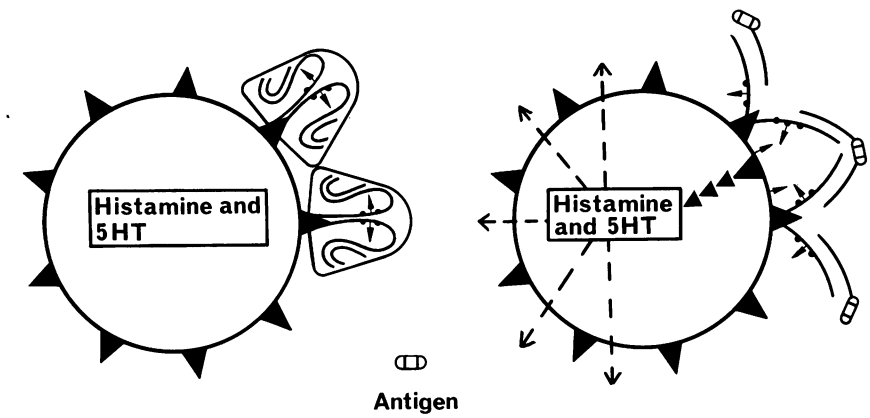

FIG. 2. Diagrammatic representation of the allosteric transition believed to occur when cells sensitized with reagin are challenged with antigen. The allosteric transition is believed to initiate a series of events ► ► which result in the release of the pharmacologically active agents, histamine and 5HT.

It is postulated that combination of antigen with the cell attached antibody results in an allosteric transition within the reaginic antibody similar to the conformational changes inducible in certain enzymes (Stanworth, 1969). This could result in the exposure of a side-chain (or chains) within the Fc region of the antibody molecule which initiates the series of enzyme reactions supposedly involved in the ultimate release of histamine and other pharmacologically active agents.

With this picture of the sequence of events, it would be possible to visualize that DSCG acts at a stage in the series of enzyme reactions triggered by the attack of the 'active site', situated in the Fc regions of the antibody molecule, on the cell surface.

The idea that DSCG acts as an enzyme inhibitor is obviously attractive but the supporting experimental evidence is inconclusive. It is true however, that DSCG inhibits mast cell degranulation and histamine release induced by the action of rattlesnake venom phos-

pholipase A on rat subcutaneous connective tissue *in vitro* (Orr & Cox, 1969). Indeed, this somewhat unexpected finding was initially taken as supportive evidence for the drug acting as an enzyme inhibitor. However, detailed *in vitro* biochemical studies using egg yolk as substrate and titrating free fatty acid as a measure of enzyme activity have shown no inhibition of phospholipase A by DSCG (Hines, Moss & Cox, unpublished observations). It would appear unlikely, therefore, that DSCG influences the phospholipase A triggered degranulation of mast cells by specifically blocking this enzyme.

There is an interesting formal relationship between the observation that inhibition of the allergic reaction occurs only when DSCG is present at the time of the antigen presentation to the sensitized cell, and the studies of Becker & Austen (1966) with organophosphorus inhibitors. Organophosphorus inhibitors such as diisopropylfluorophosphate (DFP) or the phosphonate esters are able to specifically and irreversibly inhibit a particular group of esterases, the so called 'serine esterases' (Hartley, 1960). These compounds have been shown to inhibit antigen induced release of histamine from guinea-pig lung slices and from rat peritoneal mast cells, thus implying that serine esterase is involved in these reactions (Austen & Brocklehurst, 1961; Becker & Austen, 1964; Becker & Austen, 1966). However inhibition occurs only when the inhibitor is present at the time antigen is presented to the sensitized cell (cf. DSCG).

There is no evidence to support the idea that DSCG is an esterase inhibitor and it seems most unlikely that the drug acts in the same manner as the organophosphorus inhibitors.

Drugs of the catecholamine and methylxanthine series have been shown by Lichtenstein & Margolis (1968) to inhibit the antigenically induced histamine release from human leucocytes. More recently Assem & Schild (1969) have demonstrated that a number of catecholamines also prevent antigen induced histamine release in passively sensitized human lung tissue.

Here again the one aspect of these compounds which makes them of interest is that Lichtenstein & Margolis (1968) showed that they inhibit only if present when antigen is added. That is, if the cells are pre-incubated with the inhibitor and this is then washed away before antigen is added, there is no inhibition. Evidence was also presented to suggest that both theophylline and isoproterenol operated through the adenyl cyclase 3′5′ cyclic AMP system.

Several investigations have demonstrated that DSCG does not inhibit *in vitro* histamine release from human leucocytes and the reasons for this are at present unknown. Nevertheless DSCG does inhibit *in vitro* antigen induced histamine and SRS-A release in passively sensitized human lung. Furthermore from both clinical as well as pharmacological studies there is no evidence that DSCG causes either stimulation of β adrenergic receptors *per se* or enhances stimulation of these receptors by β adrenergic agonists. Consequently, it seems unlikely that the inhibitory effect of DSCG is related to the adenyl cyclase 3′5′ cyclic AMP system.

Hence, although it is not possible at this time to specify the precise mechanism of action of DSCG in modifying reaginic antibody/antigen systems, a number of possibilities have been eliminated.

The compound does not appear, at least in the rat, to affect either the fixation of the appropriate antibody on the mast cell or the interaction of the antigen with the cell fixed antibody. It does allow, if present at the time of antigen/antibody combination, desensitization of certain tissues in the absence of the release of pharmacological mediators of ana-

phylaxis. Of special interest is the finding that once desensitization to a particular antigen has taken place in the presence of the drug the mast cells are protected even in the absence of DSCG against further attack by that antigen but not unrelated antigens.

Whilst we are hesitant to extrapolate pharmacological results obtained in an animal model to the clinical situation, these results suggest that in allergic asthmatics DSCG exerts its therapeutic effect by allowing antigen/antibody combination to occur without the release of pharmacologically active substances, so that in this manner a desensitization is brought about because the antibody is inactivated. Thus the inactivation of the sensitizing antibody by combining with the antigen during DSCG therapy may give prolonged protection even in the absence of the drug until resensitization occurs presumably by the renewed build up of sensitizing antibody on the surface of the mast cell. However, this longer term protective effect of DSCG would be expected to involve only those specific antigens to which the patient had been exposed under the protective effect of the drug.

The fact that DSCG appears to act either directly or indirectly at a stage in the sequence of events following antigen/antibody combination is therefore beneficial for two reasons; when antigen/antibody reaction takes place in the presence of the drug the release of pharmacologically active substances is inhibited and the tissues also become desensitized.

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