

RECENT DEVELOPMENTS CONCERNING THE MAST CELL AND THE MODE OF ACTION OF DISODIUM CROMOGLYCATE

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I would like to start by paying tribute to the work on mast cells of Professor Uvnäs and his group over the past 13 years. We are indebted to him for a number of techniques which we have applied in our experimental systems and which have considerably accelerated our investigations.

In studying the allergic reaction, we are dealing with a reaction that can take place in the respiratory tract, the nose or the eye. We are probably dealing with a tissue-fixed antibody, possibly IgE (4, 5). I say possibly, because there are still unresolved questions about the nature of the antibody (13). The combination of this tissue-fixed antibody with the appropriate antigen leads to the release of mediators such as histamine, 5-HT and SRS-A from mast cells (7, 14, 20). The pharmacological effects—increased capillary permeability, vasodilation, smooth muscle contraction and probably many others—are the result of this immediate-type reaction.

The activity of Lomudal in preventing immediate antigen-induced bronchospasm in experimental provocation studies in man was discovered in 1965 (1). Inhalation of DSCG before antigen challenge completely prevents any decline in lung function. After the first demonstration of activity, we were obviously very interested to find out how and where DSCG worked. In Fisons' laboratories we developed a technique in which pieces of human lung were chopped, incubated with IgE and the unfixed antibody removed by washing (Fig. 1). This gave us a model system in which we could measure the effect of various agents on the antigen-induced release of histamine

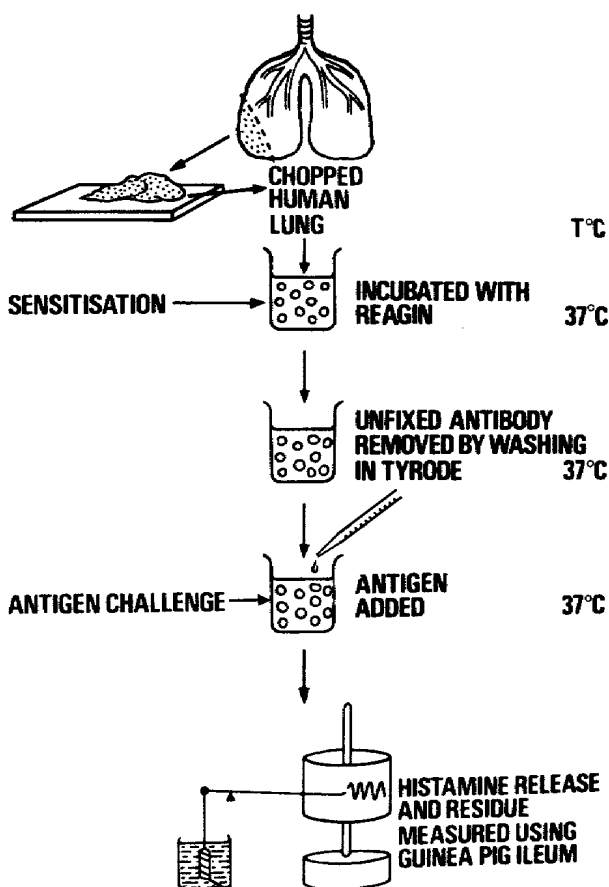
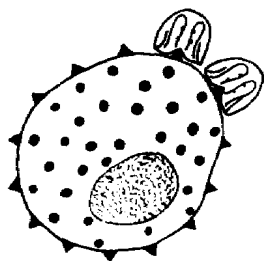
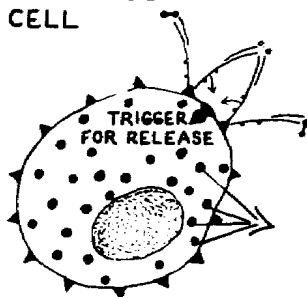


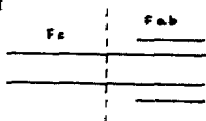
Fig. 1.

Antigen-induced histamine release from human lung *in vitro*.

and SRS-A (17). We found that DSCG inhibited this release, but only if it was present at the time of antigen challenge (16). We could find no other pharmacological activity for the drug and no actual antagonism of histamine or SRS-A.

SENSITISED
CELLCHALLENGED
CELL

ANTIBODY



ANTIGEN



Fig. 2.

Diagrammatic representation of antigen-antibody combination on sensitized cell.
(After Stanworth).

At about this time, in 1969, Dr Stanworth published his classic diagram of the immediate-type reaction occurring on the surface of the mast cell (Fig. 2) (18). If I may remind you of the details, attachment of the antibody to a receptor occurs, possibly on the outside of the cell. It may be on the inside, but I shall discuss that later. The subsequent combination of the cell-fixed antibody with antigen induces changes which activate the release process within the mast cell. We therefore became very interested in any system in which we could study the mast cell and the effect of DSCG on the release process. Some of our first studies were done in the rat using the classic PCA test. We used two different cell-fixing antibodies, IgE and IgG_a (otherwise known as 7Sγ₂). Both are capable of sensitizing mast cells and initiating the release process when combined with antigen. With DSCG we were able to show up to 100 per cent inhibition of the rat PCA

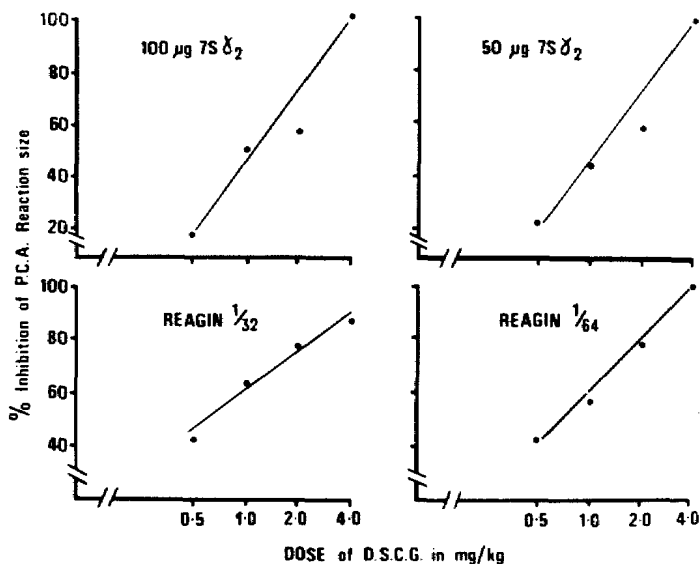


Fig. 3.

The relationship between inhibition of PCA reaction and dose of DSCG in the rat, using two types of sensitizing antibodies.

reaction with either antibody and we recorded very similar dose response curves (Fig. 3) (3, 10).

While this work was in progress we became aware of the first reports of the effectiveness of DSCG in conditions such

Histamine Release.

	Control (%)	Challenge (%)	Challenge + disodium cromoglycate (%)	Inhibition (%)
Rat reaginic system	8.7	31.0	14.9	72.2
Phospholipase A	3.4	21.5	7.3	78.4

Fig. 4.

Inhibition by DSCG of histamine release from rat subcutaneous mast cells.

Effect of Disodium Cromoglycate on the Histamine Release Induced by Compound 48/80 in Rat Peritoneal Mast Cells.

48/80 concentration	Histamine release (%)	Percentage inhibition of histamine release with DSCG at:			
		$1 \times 10^{-6} \text{M}$	$1 \times 10^{-4} \text{M}$	$2.5 \times 10^{-4} \text{M}$	$5 \times 10^{-4} \text{M}$
0 $\mu\text{g/ml}$	1.60 ± 0.03				
0.1 $\mu\text{g/ml}$	29.5 ± 0.7	26.9	12.9	59.4	59.4
0.2 $\mu\text{g/ml}$	61.2 ± 1.5	7.4	1.5	12.1	14.1
0.4 $\mu\text{g/ml}$	77.1 ± 0.8	0	6.6	4.1	16.3
1.0 $\mu\text{g/ml}$	85.1 ± 0.5	0	0	0	4.3

Fig. 5.

Effect of DSCG on histamine release from rat peritoneal mast cells induced by compound 48/80.

as exercise-induced asthma, where there was no obvious allergic stimulus (2). This drew our interest to earlier experimental work by Professor Uvnäs, which had suggested that degranulating agents such as compound 48/80 could open mast cells in a selective rather than a lytic manner and that this process might be comparable to antigen-induced release. The possible parallel with the clinical situation was clear and we decided to study the effect of degranulating agents on mast cells. Initially, we used phospholipase A, one of the

Effect of Disodium Cromoglycate on the in vivo Degranulation of Mast Cells Induced by Compound 48/80.

48/80 concentration ($\mu\text{g/site}$)	Percentage mast cell degranulation control	Percentage inhibition of mast cell degranulation with DSCG at:	
		10 mg/kg	100 mg/kg
0.125	37	65 (<.05)	73 (<.02)
0.25	56	77 (<.001)	57 (<.01)
0.5	82	65 (<.001)	34 (<.02)

P values in brackets are those compared with controls.

Fig. 6.

Effect of DSCG on the degranulation of rat mast cells *in vivo* induced by compound 48/80.

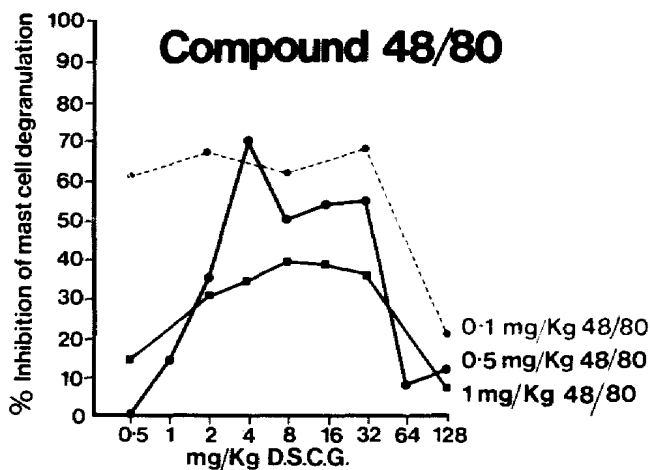


Fig. 7.

Inhibition by DSCG of compound 48/80-induced mast cell degranulation.

histamine-releasing agents studied by Professor Uvnäs. We found that DSCG had a very marked protective effect on rat connective tissue mast cells against phospholipase A. The results in terms of histamine release were very similar to those with the IgE system—78 per cent protection in the phospholipase system and 72 per cent in the IgE model (Fig. 4) (8). This protection was seen first *in vitro*, but there are limitations to an *in vitro* system and we spent a considerable time developing *in vivo* methods for studying the effect of degranulators. We were successful with another degranulator, compound 48/80. A protective effect had been shown at various concentrations *in vitro* (Fig. 5) and we showed a similar effect *in vivo* when the degranulator was administered intravenously (Fig. 6) (11) and (Fig. 7) (9). We went on to study other degranulators, polymixin and dextran, and again found a protective effect of DSCG *in vivo*, to a maximum of 70–80 per cent (9). Again, we observed the bell-shaped dose response curve (Figs. 8 and 9). We have as yet no definite

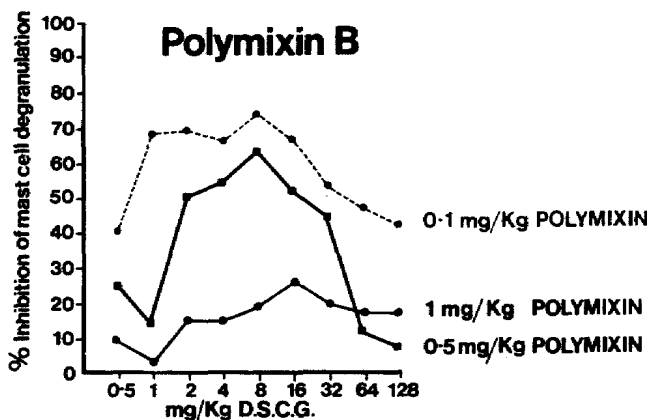


Fig. 8.

Inhibition by DSCG of polymixin B-induced mast cell degranulation.

explanation of this, although we have a hypothesis. One possible explanation is to postulate that the DSCG molecule must attach to the cell at two points in order to be active. At high concentrations the probability of this divalent attachment would decrease and monovalent attachment would become more likely. This would lead to a reduction in activity. This is one of our current hypotheses. I should perhaps mention that we have seen no evidence of a bell-shaped curve in provocation tests in man, so that its clinical relevance is obscure.

To summarize, then, we have demonstrated a protective effect of DSCG on the mast cell in systems where the releasing factor is immunological, such as IgE and IgG_a sensitization and antigen challenge, and non-immunological, such as degranulating agents. I should add that we have also studied agents which have a lytic effect and cause rupture of the mast cell, such as Triton X-100. DSCG has no protective effect against these agents. It is effective in systems where there is a pathway of selective release from the mast cell, whether the factor activating this pathway is immunological or non-immunological.

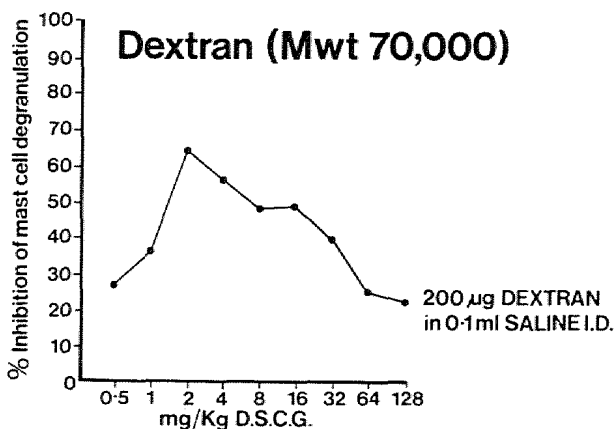


Fig. 9.

Inhibition by DSCG of dextran-induced mast cell degranulation.

I should like now to explain to you some of the mode-of-action studies we have carried out in rats. We had an obvious interest in finding out how DSCG works. For example, if the drug had any effect on antibody formation, this could be disastrous in other diseases. We used first an *in vitro* rat PCA system, in which we removed rat subcutaneous connective tissue, sensitized it with antibody and washed off the excess (12). We could then challenge with antigen and assay histamine release (Fig. 10). We found that DSCG had no effect whatever on the attachment of antibodies to the mast cell, but that it did prevent histamine release if it was present at the time of antigen challenge. A possible explanation of these experimental results was that DSCG might be preventing the combination of antibody and antigen.

We investigated this possibility by washing off excess antigen after the first challenge *with* DSCG present and then rechallenging with antigen in the *absence* of DSCG. No further histamine release occurred in the second challenge and this was reasonably indicative evidence that antigen-antibody

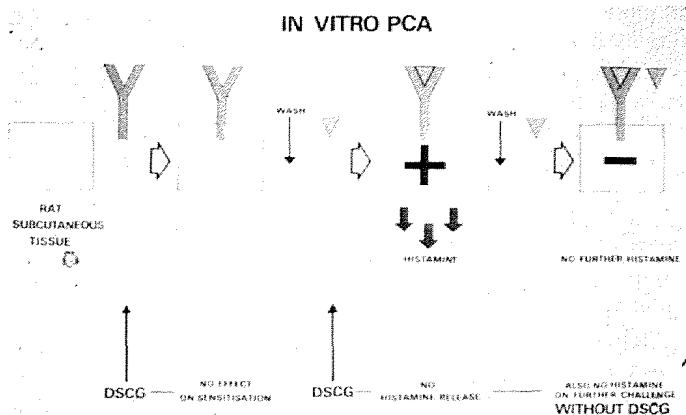


Fig. 10.

The effect of DSCG on the PCA reaction *in vitro*.

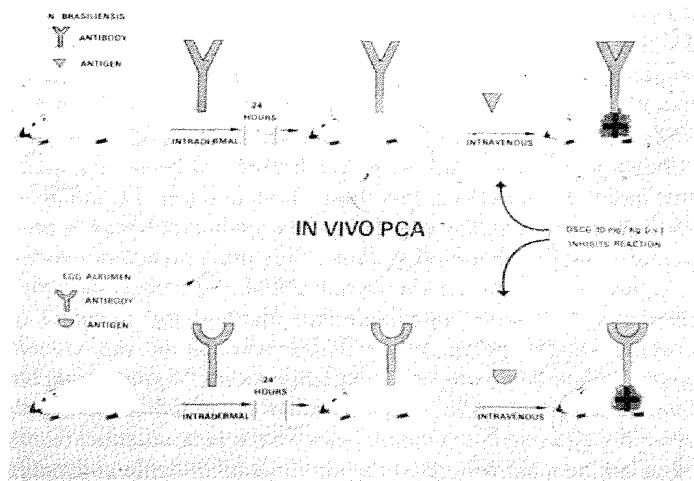


Fig. 11.

The PCA reaction in the rat *in vivo*, using two different antibodies.

had taken place at the first challenge. The experiment was not, however, wholly satisfactory, because it was impossible to have controls throughout the sequence and because it was purely *in vitro*.

We therefore went on to develop an *in vivo* system in which we injected two antigenically distinct IgE antibodies into a rat at the same time. The animal was thus sensitized to two different antigens, both of which are capable of causing a full PCA reaction on challenge. The antibodies used were those to *Nippostrongylus brasiliensis*, a helminth, and to egg albumen. It could easily be shown that sensitization to both has occurred by challenge at 24 hours (Fig. 11). We assumed that both antibodies were fixed to the same sites. The reaction sequence was as follows. First we divided the doubly-sensitized animals into two groups and challenged one group with the *Nippostrongylus* antigen in the presence of DSCG and one group in the absence of DSCG. The dose of DSCG was carefully titred so that none was left at 4 hours. As expected, the normal PCA reaction was seen only in the group not treated with DSCG. After a period of 4 hours, the groups were subdivided and, without DSCG cover, half of each was rechallenged with *Nippostrongylus* antigen and half with egg albumen.

The group which had initially given a reaction to *Nippostrongylus* did not react to either antigen. The group which had initially been challenged under DSCG cover did not react to the second *Nippostrongylus* challenge but did give a positive albumen reaction (Fig. 12). Our interpretation of this sequence is that during the initial challenge, antigen-antibody union occurs in both groups, independent of the presence of DSCG. DSCG prevents the PCA reaction in the treated group. When this group is rechallenged with *Nippostrongylus*, there is no reaction because the specific antibodies have been utilised by the primary challenge. There is a reaction to the other antigen, showing that the system is still viable and that a positive PCA reaction can occur. The failure of the group who responded to the initial *Nippostrongylus* challenge to

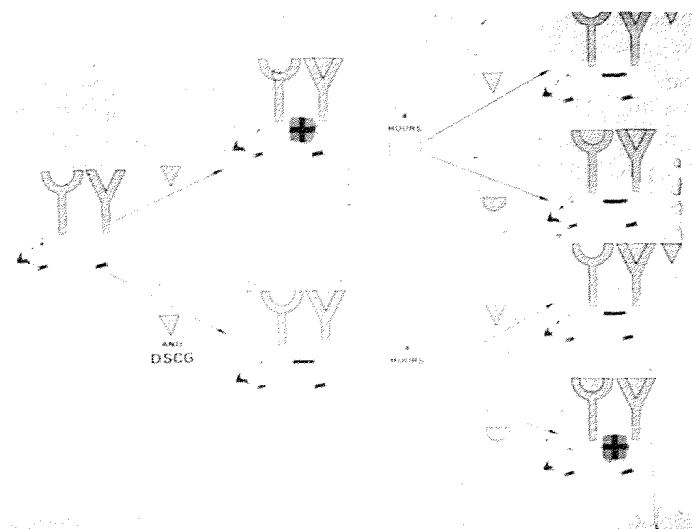


Fig. 12.

The effect of DSCG on the two-antibody rat PCA system *in vivo*.

respond to either antigen at the second challenge is probably due to some decay factor in the degranulation process. It may well take some time after the first degranulation for the mast cells to be capable of the further degranulation necessary for a PCA reaction. This may seem a very complicated explanation, but please remember that we were trying to demonstrate conclusively in a controlled study *in vivo* that DSCG did not affect antigen-antibody union.

The next question to be resolved was the location within the mast cell of the site of reactivity. Our approach to this was to use a very high titre of rat IgE to sensitize rat subcutaneous connective tissue mast cells, to fix the cells and then to challenge with fluorescently labelled antigen. The site of antibody-antigen union was located by the fluorescence after challenge. We expected to find the fluorescence localized on the outside of the mast cell, because most of the other evidence at that

time indicated that the cell membrane was the site of union. We found, in fact, that while some of the fluorescence was on the cell membrane, there was also definite fluorescence within the cell. This was in rather a granular form. To confirm this apparent intracellular location of antigen-antibody union, we disrupted the mast cells with distilled water and using the same tissue we stained for mast cell granules with toluidine blue and looked for fluorescence. There was an exact coincidence between toluidine blue-positive and fluorescent material. This would indicate that at least part of the antigen-antibody union takes place in close proximity to the mast cell granules.

The very high turnover rate of cell membrane is relevant to the question of antibody attachment to the mast cell. If the attachment of IgE antibody was purely to the cell membrane,

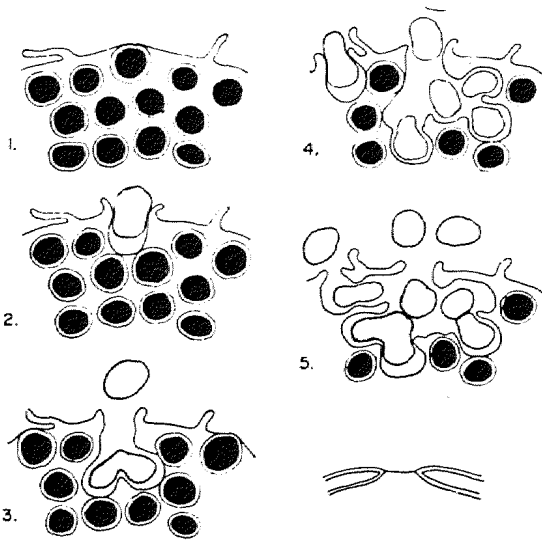


Fig. 13.

Schematic diagram of granule expulsion (1, 2, 3) and labyrinth formation (4, 5) in degranulating mast cell (after Uvnäs).

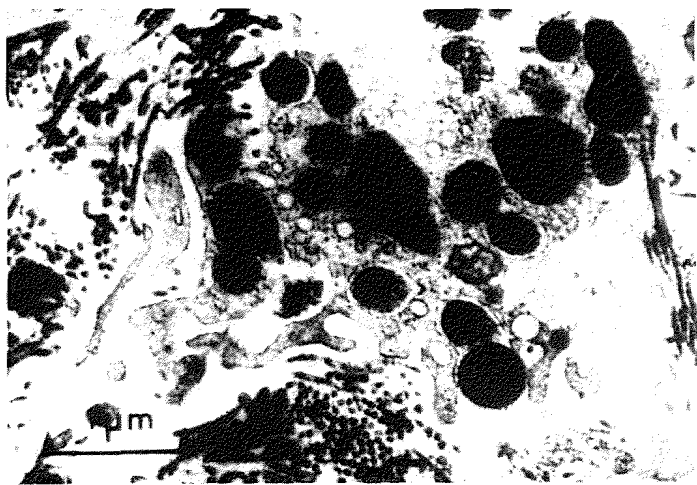


Fig. 14.

Human nasal mast cell, demonstrating peripheral granule extrusion and perigranular labyrinth formation.

it is difficult to explain the very long period of attachment of IgE to mast cells. One would have to postulate some system of transfer of IgE from existing to newly formed membrane. An intracellular locus for IgE could explain the long period of attachment.

The studies of Professor Uvnäs and others, notably Lagunoff in America, have done much to elucidate the actual mechanism of mast cell degranulation (6, 15). The sequence of events seems to be that peripheral mediator-containing granules are the first to be extruded from the cell. At the same time a system of labyrinths develops which exposes granules still within the cell to the extracellular environment (Fig. 13). As a result, these granules within the labyrinth can release mediators by a simple process of ionic exchange. The final stage is extensive labyrinth formation within the cell, with many of the intracellular granules releasing their contents. At

this stage, it is very difficult to decide by light microscopy whether a cell has degranulated or not.

So far, I have been talking about rat mast cells, but I would now like to present some of our electron micrograph studies of human mast cells. First of all, I would like to show you a mast cell from human nasal mucosa, which shows stages in the degranulation process (Fig. 14). Peripheral granules have been extruded, labyrinths are forming and granules are breaking down within the labyrinths and releasing their contents (19). The process of granule extrusion is known to be an energy-consuming process. It is also a rapid process—less than 30 seconds for rat mast cells—and it is likely to result from some motive force.

Our current thinking is that the membrane changes and

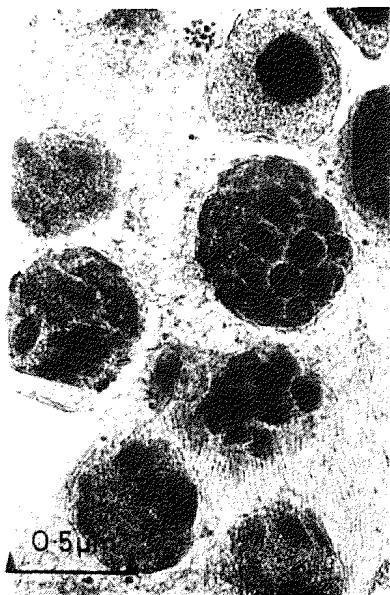


Fig. 15.

Human mast cell showing bands of microfilaments.

granule expulsion are very closely related to the structures with the mast cells known as microfilaments. On high-power electron micrographs, these may be seen as a mass of very small filaments (Fig. 15). One of the characteristics of human mast cells is the web of these contractile microfilaments that they contain (19). The filaments are much less easy to see in rat mast cells. Another characteristic of human mast cells is that the granules contain a variety of inclusions, best described as whorls and scrolls. The role of these structures is not yet known.

Our theory is that contractions of the microfilaments play an important part in the membrane changes and granule expulsion. These changes are energy-consuming and can be prevented by metabolic inhibitors. In addition, we have shown that DSCG inhibits completely all the events in the sequence. In the presence of DSCG, none of the structural changes occur; this would indicate that the drug is acting at a very early stage after antigen-antibody union. It is possible that this union leads either to an influx of calcium ions or to an endogenous liberation of calcium within the cell. The increased calcium concentration may activate a series of enzyme pathways, probably including an ATP-ase system, to provide the energy necessary for microfilament contraction, granule expulsion and labyrinth formation. Over the next few years I hope we will learn a great deal about this process. I think we will find that the initial reaction of the immediate allergic response takes place on the cell membrane and then cascades into the cell. I would like to think that we will find that DSCG is a very effective inhibitor of that initial event.

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DISCUSSION

A. Jenssen: I would like to ask if Dr Orr thinks that the activity of Lomudal is due to calcium binding effect. I have found that it forms gelatinous precipitates with calcium ions over a pH range from 1-12.

S. Orr: What concentrations were you using?

A. Jenssen: Five percent.

S. Orr: That is extremely high. We normally use only microgram amounts of Lomudal in our experiments. At these concentrations there is no evidence of calcium binding. Certainly you get gelatinous precipitates at high concentrations but this does not happen with low concentrations. In addition, the drug cannot be acting just as a calcium chelator, because other agents like EDTA, which chelate calcium very well, have no effect on the PCA reaction *in vivo*, unless lethal doses are used. They do have an effect on the PCA reaction *in vitro*. To think of the drug as a calcium chelator also gives no indication of how it can be so specific for mast cells and have no effect on other cellular calcium-dependent systems.

P. Andersson: Do the granules outside the mast cell have the same electron density as those inside?

S. Orr: They tend not to. In human mast cells, the substructure of the granule, the whorls and scrolls, disappears before it leaves the cell.

T. Berg: Do you think it possible that one allergen can induce partial degranulation of a mast cell and another allergen can induce the expulsion of the remainder of the granules?

S. Orr: I think it is perfectly possible to have two antigenically distinct antibodies present on one mast cell and to induce a localized, partial release of granules. Some of your work with degranulators has shown that localized release is possible with controlled challenge.

H. Colldahl: In our work with basophils, we have been un-

able to prevent antigen-induced histamine liberation with Lomudal. Do you have any experience with basophils?

S. Orr: The extent of the similarity between basophils and mast cells is an interesting subject. They are totally different in ultrastructure—human basophils have none of the inclusions of the mast cell. I agree that Lomudal does not seem to inhibit histamine release, but I think you have to bear in mind the recent work on release of platelet-activating factors from basophils and make sure that you are observing a specific IgE reaction.

J. Pepys: I found your suggestion that IgE molecules might be located within the cell very interesting. If this were so, it would be a unique situation of an immunoglobulin in a non-immunoglobulin producing cell. An alternative explanation of the fluorescence on the granules could be that they take up antibody after liberation from the cell. Your demonstration of intracellular fluorescence could be explained if you were looking at the top of the cell rather than a section. Perhaps you could investigate the possible uptake of IgE by granules after liberation?

S. Orr: I agree with your comments.

B. Uvnäs: Does Lomudal have any inhibitor effect in other enzyme systems? One possibility would be prostaglandin synthetase. I would also like to ask you about your electron micrographs of elastic fibres. How do you know they are fibres and how do you know they are elastic?

S. Orr: We have never seen any specific enzyme inhibition from Lomudal at doses at which it exerts its anti-allergic effect although we have studied a number of systems including prostaglandin synthetase. Your comment on the fibres is well taken. We are really extrapolating from our knowledge of similar fibres in other pharmacological situations and saying that these fibres in mast cells bear a close resemblance. We have not shown that they are elastic fibres, but they conform to the dimensions and configuration of known elastic fibres to within a few ångströms.

B. Arner: I have two questions. Do you think that the

histamine release that you see with dextran in the rat bears any relation to the allergic side-effects of dextran in man? Could reactions to other foreign compounds involve histamine release from mast cells?

S. Orr: I think that reactions to dextran in man bear no relation to those with rat mast cells. In man it is a true hypersensitivity shown by only a very few individuals. In our strain of rat, all animals will show mast cell degranulation in response to dextran.

G. Johansson: Does Lomudal prevent the release caused by anti-IgE or performed allergen-reagin complexes?

S. Orr: Yes, it does prevent anti-IgE induced release. We have done this with human lung with mono-specific anti-IgE sera and also with a reverse PCA reaction in the rat as well, using rat anti-IgE. We have not looked at performed complexes but it is difficult to decide on the experimental system to use. Should we use the monkey, which is not susceptible to Lomudal, or should we use the skin where we know the drug has apparently no effect?

J. Pepys: Further to Professor Uvnäs's question on the microfilaments, I remember that there was a report from Prague some years ago that muscle actomyosin could be sensitized with antibody and that subsequent antigen challenge elicited a contraction. If Lomudal could be shown to have any direct effect on the contraction of mast cell fibres, this would be relevant to your argument for intracellular antibody.

S. Orr: At present we are trying to extract individual fibres from skeletal muscle and also smooth muscle and to study the effect of sensitization. We think this could be a very interesting approach to the problem.

B. Uvnäs: There have been attempts to isolate the elastic fibres from leucocytes. Perhaps you might use the same techniques for mast cells.

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