# Stimulation of mast cells leads to cholesterol accumulation in macrophages *in vitro* by a mast cell granule-mediated uptake of low density lipoprotein

(heparin proteoglycan/phagocytosis/lipoprotein metabolism/atherosclerosis)

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ABSTRACT     The uptake of low density lipoprotein (LDL) by cultured mouse macrophages was markedly promoted by isolated rat mast cell granules present in the culture medium. The granule-mediated uptake of LDL enhanced the rate of cholesteryl ester synthesis in the macrophages, the result being accumulation of cholesteryl esters in these cells. Binding of LDL to the granules was essential for the granule-mediated uptake of LDL by macrophages, for the uptake process was prevented by treating the granules with avidin or protamine chloride or by treating LDL with 1,2-cyclohexanedione, all of which inhibit the binding of LDL to the granules. Inhibition of granule phagocytosis by the macrophages with cytochalasin B also abolished the granule-mediated uptake of LDL. Finally, mouse macrophage monolayers and LDL were incubated in the presence of isolated rat serosal mast cells. Stimulation of the mast cells with compound 48/80, a degranulating agent, resulted in dose-dependent release of secretory granules from the mast cells and a parallel increase in cholesteryl ester synthesis in the macrophages. The results show that, in this *in vitro* model, the sequence of events leading to accumulation of cholesteryl esters in macrophages involves initial stimulation of mast cells, subsequent release of their secretory granules, binding of LDL to the exocytosed granules, and, finally, phagocytosis of the LDL-containing granules by macrophages.

The cytoplasm of rat serosal mast cells is filled with specific organelles, the secretory granules. The granules are composed of a heparin proteoglycan matrix in which the other components of the granules are embedded (1). On antigen challenge, the mast cells are activated and extrude their granules into the extracellular space, where the soluble components of the granules, such as histamine, are released and diffuse away to exert their functions in the allergic reactions (1). In contrast, the major granule components, consisting of neutral proteases and the heparin proteoglycan, remain tightly bound to each other, so forming extracellularly located insoluble granule "remnants." We have recently observed that such extracellular mast cell granules may specifically interact with low density lipoprotein (LDL), in that LDL is bound to the heparin proteoglycan of the granules (2). We now extend this observation and report that binding of LDL to the granules results in marked enhancement of its uptake by cultured mouse macrophages *in vitro* due to phagocytosis of the LDL-containing granules.

## MATERIALS AND METHODS

**Materials and Animals.** Na$^{125}$I (13–17 mCi/$\mu$g; 1 Ci = 37 GBq) and [1-$^{14}$C]oleic acid (50–60 mCi/mmol) were purchased from Amersham International. Avidin, bovine serum albumin, soybean trypsin inhibitor, protamine chloride,

cytochalasin B, and compound 48/80 were obtained from Sigma. Phenylmethylsulfonyl fluoride (PhMeSO$_2$F) was purchased from Boehringer Mannheim. Eagle's basal medium with Earle's salts (EBME) with 20 mM Hepes was purchased from Flow Laboratories. Plastic Petri dishes (35 × 10 mm) were obtained from Falcon. 1,2-Cyclohexanedione was purchased from Fluka. Male Wistar rats (300–500 g) and female NMRI mice (25–35 g) were obtained from Orion (Espoo, Finland).

**Isolation of Mouse Peritoneal Macrophages.** Macrophages were harvested from unstimulated NMRI mice in Dulbecco's phosphate-buffered saline containing 2 mg of bovine serum albumin per ml as described (3). The peritoneal cells were resuspended in medium A (EBME containing 20% heat-inactivated fetal calf serum, 100 international units of penicillin per ml, and 100 $\mu$g of streptomycin per ml). Cells (3 × 10$^6$) were seeded into plastic Petri dishes (35 × 10 mm) and incubated in a humidified CO$_2$ (5% CO$_2$ in air) incubator at 37°C for 1 hr. Nonadherent cells were then rinsed off with EBME (3 × 2 ml), and the macrophage monolayers were incubated in 1 ml of medium A overnight before use in experiments. For the experiments the dishes were washed twice with 2 ml of EBME and the medium was replaced with 1 ml of medium B (EBME containing 10 mg of bovine serum albumin per ml, 100 $\mu$g of soybean trypsin inhibitor per ml, and 100 international units of penicillin per ml).

**Isolation of Rat Mast Cells and Mast Cell Granules.** Mast cells and mast cell granules were isolated as described (4). The quantity of granules is expressed in terms of granule protein. PhMeSO$_2$F-treated granules were obtained by incubating 50 $\mu$g of granules in 1 ml of buffer consisting of 150 mM NaCl, 5 mM Tris·HCl (pH 7.4), 10 mg of bovine serum albumin per ml, and 250 $\mu$g of PhMeSO$_2$F per ml for 15 min at 37°C. After incubation the granules were washed twice with the buffer and finally resuspended in medium B. Avidin- and protamine-treated granules were obtained by incubating 50 $\mu$g of granules for 5 min at 4°C in 100 $\mu$l of buffer (150 mM NaCl/5 mM Tris·HCl, pH 7.4) containing 1.5 mg of avidin per ml or 250 $\mu$g of protamine chloride per ml, respectively. After incubation the granules were washed twice with the buffer and finally resuspended in medium B. $^{35}$S-labeled granules ($^{35}$S-granules) were obtained by incubating isolated mast cells *in vitro* with [$^{35}$S]sulfate as described (2). The degradation of $^{35}$S-granules by mouse macrophages was determined by gel filtration on a Sepharose 4B column (Pharmacia) (5). In the degradation experiments, $^{35}$S-granules were incubated with macrophage monolayers as described in the legend to Table 1. After incubation the medium was removed and NaCl was added to give a final concentration of 1 M. One milliliter of the medium was then applied to a Sepharose 4B column (1 ×

Abbreviations: LDL, low density lipoprotein; PhMeSO$_2$F, phenyl-methylsulfonyl fluoride.
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20 cm), and the column was eluted with 1 M NaCl containing 0.02% $NaN_3$ at a flow rate of 3.4 ml/hr at room temperature, and the fractions were measured for their $^{35}S$ radioactivity. The material eluting in the position corresponding to the total volume of the column represented the low-molecular-weight degradation products of $^{35}S$-granules (5).

**Preparation and Iodination of Lipoproteins.** Human LDL ($\rho$ = 1.019–1.050) was fractionated by sequential ultracentrifugation and iodinated by the iodine monochloride method, as described (4). Cyclohexanedione-LDL was prepared by treatment of LDL with 1,2-cyclohexanedione as described (6). The concentration of each LDL preparation is expressed in terms of its protein concentration. For experiments, the labeled LDL was diluted with unlabeled LDL to give the specific activities indicated in the figure and table legends.

**Proteolytic Degradation of $^{125}I$-Labeled LDL ($^{125}I$-LDL) and Incorporation of [$^{14}C$]oleate into Cholesteryl Esters by Macrophages.** In a standard degradation assay, each dish received the indicated amounts of $^{125}I$-LDL and PhMeSO$_2$F-treated granules. After incubation at 37°C for the indicated times, the medium of each dish was removed and treated with trichloroacetic acid (4). Blank values were obtained by incubating $^{125}I$-LDL and granules in dishes containing no cells. The incorporation of [$^{14}C$]oleate into cholesteryl esters by macrophages was determined by the method of Brown *et al.* (7). In a standard assay, each dish received the indicated amounts of LDL and granules and [$^{14}C$]oleate-albumin to give a final oleate concentration of 200 $\mu$M. After incubation for the indicated times, the lipids were extracted *in situ* with hexane/isopropyl alcohol (3:2), and the cholesteryl[$^{14}C$]oleate was isolated by thin-layer chromatography (7). The cells in the dishes were then dissolved in 0.2 M NaOH and aliquots were removed for protein determination.

**Other Assays.** Histamine was determined by fluorometry (4). The cellular content of esterified cholesterol was measured by gas/liquid chromatography (8). Protein was determined by the procedure of Lowry *et al.* (9), with bovine serum albumin as standard. $^{125}I$-LDL binding to mast cell granules was determined as described (2), except that the binding assay was conducted in medium B.

## RESULTS

We first studied the effect of mast cell granules on the ability of cultured mouse macrophages to degrade $^{125}I$-LDL. Since the mast cell granules themselves also degrade $^{125}I$-LDL (4), their proteolytic activity had to be inhibited during incubation with $^{125}I$-LDL and macrophages. Complete inhibition of LDL degradation by the granules was achieved if the granules were treated with PhMeSO$_2$F before addition to the culture medium ("PhMeSO$_2$F-granules"; see *Materials and Methods*).

When mouse macrophages were then incubated with $^{125}I$-LDL (50 $\mu$g/ml) and increasing quantities of PhMeSO$_2$F-granules, $^{125}I$-LDL degradation by the macrophages was found to be a function of granule concentration (Fig. 1A). At the maximal quantity of granules used (20 $\mu$g of granule protein) the rate of LDL degradation was 8-fold the rate without granules. When the quantity of granules was kept constant (20 $\mu$g per assay), increasing the concentration of $^{125}I$-LDL led to an increase in the rate of degradation, with evidence of saturation (Fig. 1B). By contrast, in the absence of granules, the degradation of $^{125}I$-LDL was not saturable but increased linearly with increasing concentrations of $^{125}I$-LDL. Extracellular degradation of LDL by macrophage-derived proteolytic enzymes was excluded by demonstrating the absence of $^{125}I$-LDL degradation in preconditioned medium obtained by incubating macrophages for 24 hr with PhMeSO$_2$F-granules alone (data not shown).

Degradation of $^{125}I$-LDL by the macrophages was consistent with cellular uptake of the labeled lipoprotein. As an index of uptake, we measured the incorporation of [$^{14}C$]oleate into cholesteryl[$^{14}C$]oleate in macrophages, an intracellular process known to correlate with the amount of cholesterol entering the cell (3). In these experiments the PhMeSO$_2$F treatment of granules could be omitted (the stimulation of cholesteryl ester synthesis in macrophages by PhMeSO$_2$F-treated and untreated granules was identical). Fig. 2A shows that macrophages incubated with LDL in the absence of granules incorporated only small amounts of [$^{14}C$]oleate into cholesteryl[$^{14}C$]oleate. However, addition of granules to the incubation medium led to marked stimulation of cholesteryl[$^{14}C$]oleate synthesis in macrophages. As the granule concentration increased, the rate of cholesteryl ester synthesis increased in a fashion similar to that found for the granule-mediated stimulation of $^{125}I$-LDL degradation (see Fig. 1A). In macrophages incubated with LDL and granules for varying lengths of time, the rate of cholesteryl ester synthesis showed an initial lag phase of about 4 hr and thereafter continued at a linear rate for at least 21 hr (Fig. 2B). At 21 hr, the amount of cholesteryl[$^{14}C$]oleate in macrophages incubated in medium containing granules was 10-fold the amount in macrophages incubated in medium devoid of granules. In additional experiments we showed that the granule-dependent stimulation of cholesteryl ester synthesis could be inhibited by such lysosomotropic agents as chloroquine (75 $\mu$M) and ammonium chloride (15 mM) (data not shown). Thus, the results are consistent with a model for lysosomal hydrolysis of the cholesteryl esters of LDL with subsequent reesterification of the cholesterol in the cytoplasm, a model that parallels the pathway described in macrophages incubated with acetylated LDL (3).
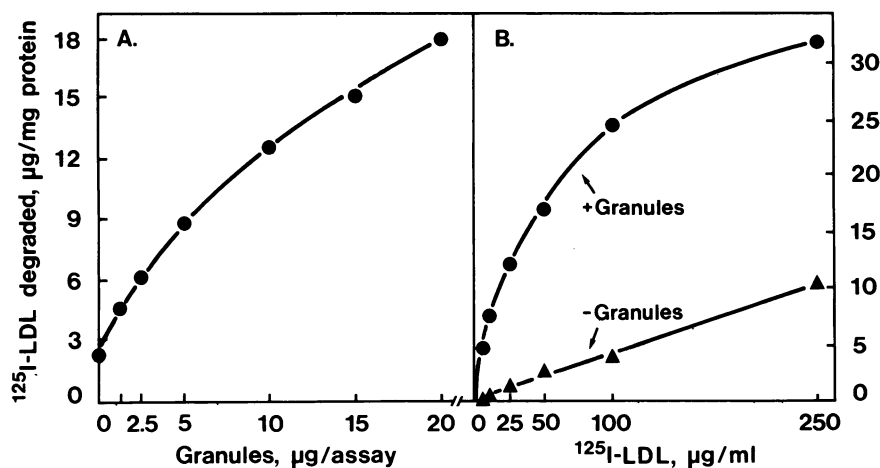


FIG. 1. Degradation of $^{125}I$-LDL in macrophages as a function of the quantity of granules (A) and the concentration of $^{125}I$-LDL in the presence of granules. (A) Each macrophage monolayer received 1 ml of medium B containing 50 $\mu$g of $^{125}I$-LDL per ml (40 cpm/ng) and the indicated amounts of granules. (B) Each macrophage monolayer received 1 ml of medium B containing 20 $\mu$g of granules (●) and the indicated concentrations of $^{125}I$-LDL (45 cpm/ng). In the control experiment no granules were added (▲). After incubation at 37°C for 18 hr, the amount of $^{125}I$-labeled trichloroacetic acid-soluble material in the medium was determined.
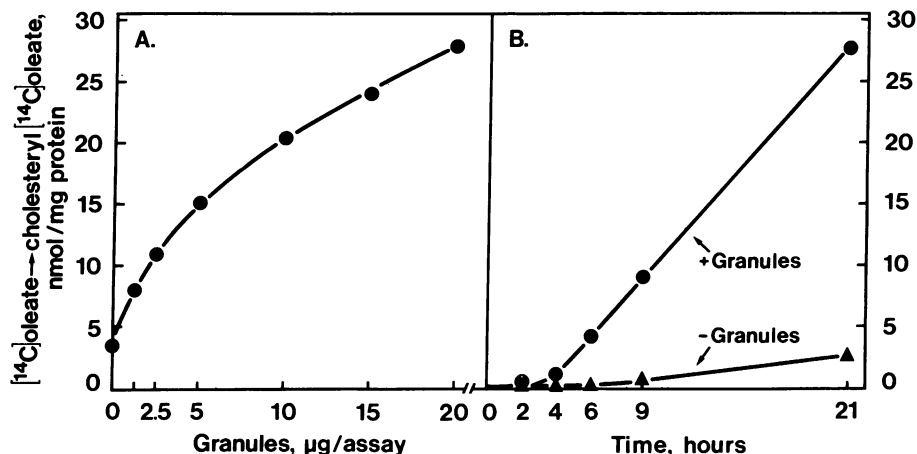
FIG. 2. Formation of cholesteryl esters in macrophages incubated with granules and LDL. (*A*) Each macrophage monolayer received 1 ml of medium B containing 50 $\mu$g of LDL per ml, 200 $\mu$M [$^{14}$C]oleate-albumin (9600 dpm/nmol), and the indicated amounts of granules. (*B*) Each macrophage monolayer received 1 ml of medium B containing 50 $\mu$g of LDL per ml, 200 $\mu$M [$^{14}$C]oleate-albumin (12,500 dpm/nmol), and 20 $\mu$g of granules (●). In the control experiment no granules were added (▲). After incubation at 37°C for 18 hr (*A*) or for the indicated times (*B*), the cellular content of cholesteryl[$^{14}$C]-oleate was determined.

The granule-mediated stimulation of cholesteryl ester synthesis in macrophages incubated with LDL was corroborated by measuring the content of cholesteryl esters in the macrophages. Macrophages incubated with LDL (50 $\mu$g/ml) alone for 18 hr contained 1.8 $\mu$g of cholesteryl esters per mg of protein (mean of triplicate incubations), whereas macrophages incubated with LDL and granules (20 $\mu$g per assay) for the same period of time contained 45 $\mu$g of cholesteryl esters per mg of cell protein—i.e., a content of cholesteryl esters higher by 25-fold. In the same experiment we also measured the rate of cholesteryl ester synthesis and found a 22-fold increase in the amount of cholesteryl[$^{14}$C]oleate in macrophages incubated with LDL and granules.

Further evidence for the essential role of mast cell granules as mediators of the uptake of LDL by macrophages was obtained by using cytochalasin B, an inhibitor of the phagocytic uptake of particles by macrophages (10). Incubation of mast cell granules with macrophages results in phagocytosis of the granules, a process that can be quantified by measuring the rate of intracellular degradation of $^{35}$S-granules (11). We were able to show that addition of 10 $\mu$g of cytochalasin B per ml to the macrophage culture inhibited granule phagocytosis by 85% and that a similar concentration of cytochalasin B was able to inhibit

the granule-mediated stimulation of cholesteryl ester synthesis in macrophages by 98%.

We have previously demonstrated that mast cell granules are able to bind LDL efficiently (2). Inasmuch as the phagocytosis of mast cell granules was now shown to be a prerequisite for the granule-mediated uptake of LDL by macrophages, we reasoned that binding of LDL to the granules would be crucial for the granule-mediated uptake of LDL. To test this hypothesis, we either treated mast cell granules with avidin or protamine chloride, two compounds that competitively inhibit the binding of LDL to mast cell granules (2), or treated LDL with cyclohexanedione to yield a modified LDL that is unable to bind to the granules (2). As shown in Table 1, the binding of $^{125}$I-LDL to granules treated with avidin was only 18% of the value found for control granules (left column). When such avidin-treated granules were then added to macrophage monolayers containing LDL in the incubation medium, the granule-mediated stimulation of cholesteryl ester formation was only 11% of the stimulatory effect of control granules (middle column). Pretreatment of the granules with protamine chloride also strongly inhibited LDL binding to the granules and the ability of the granules to stimulate cholesteryl ester formation in the

Table 1. Effect of mast cell granule pretreatments on binding of LDL to the granules, cholesteryl ester formation in macrophages, and degradation of the granules by macrophages

| | | Experiments with macrophages | |
|---|---|---|---|
| Pretreatment of granules | Binding of $^{125}$I-LDL to granules, ng/$\mu$g of granule protein | [$^{14}$C]Oleate incorporation into cholesteryl esters,* nmol/mg of cell protein | Formation of $^{35}$S-granule degradation products,† dpm |
| None | 143  (100) | 45   (100) | 1530 (100) |
| Avidin | 25   (18) | 5.1   (11) | 1760 (114) |
| Protamine | 9.3   (7) | 6.7   (15) | 1910 (125) |

Mast cell granules were first treated with avidin or protamine chloride. The abilities of untreated and avidin- or protamine-treated granules to bind $^{125}$I-LDL were tested by using the granule binding assay (2). The assay was conducted in 100 $\mu$l of medium B containing 3 $\mu$g of untreated or avidin- or protamine-treated granules and 100 $\mu$g of $^{125}$I-LDL per ml (67 cpm/ng). After incubation at 0°C for 5 min, the amount of $^{125}$I-LDL bound to the granules was determined (left column). Then macrophage monolayers received 20 $\mu$g of either untreated or avidin- or protamine-treated granules in 1 ml of medium B containing 50 $\mu$g of LDL per ml and 200 $\mu$M [$^{14}$C]oleate-albumin (13,500 dpm/nmol). After incubation at 37°C for 18 hr, the cellular content of cholesteryl[$^{14}$C]oleate was determined (middle column). Granule degradation by macrophages was measured with $^{35}$S-granules. Each macrophage monolayer received 35 $\mu$g of untreated or avidin- or protamine-treated $^{35}$S-granules (15,800 dpm) in 1 ml of medium B. After incubation at 37°C for 24 hr, the degradation of $^{35}$S-granules by macrophages was determined (right column). Each value in the left and middle columns is the mean of triplicate incubations, and each value in the right column represents the result of a single incubation. Numbers in parentheses represent percentages of control values.

*A value of [$^{14}$C]oleate incorporated into cholesteryl esters (3.3 nmol/mg of protein) in macrophage monolayers incubated with LDL alone has been subtracted from the values obtained with LDL and granules.

†Blank values obtained by incubating $^{35}$S-granules in dishes containing no cells have been subtracted from the results.

Table 2. Effect of cyclohexanedione treatment of LDL on binding of LDL to mast cell granules and on granule-mediated stimulation of cholesteryl ester synthesis in macrophages

| LDL preparation | Inhibition of binding of $^{125}$I-LDL to granules by unlabeled LDL preparations,* % | [$^{14}$C]Oleate incorporation into cholesteryl esters, nmol/mg of cell protein | |
|---|---|---|---|
| | | With granules | Without granules |
| LDL | 95 | 28 (100) | 1.1 (100) |
| Cyclohexanedione-LDL | 23 | 3.0 (11) | 1.0 (91) |

The abilities of LDL and cyclohexanedione-LDL to inhibit $^{125}$I-LDL binding to mast cell granules were tested by using the granule binding assay (2). The assay was conducted in 100 μl of medium B containing 3 μg of isolated mast cell granules, 20 μg of $^{125}$I-LDL per ml (158 cpm/ng), and 500 μg of LDL or cyclohexanedione-LDL per ml. After incubation at 0°C for 5 min, the amount of $^{125}$I-LDL bound to granules was determined (left column). In experiments with macrophages (middle and right columns), each macrophage monolayer received 20 μg of granules in 1 ml of medium B containing 200 μM [$^{14}$C]oleate-albumin (11,500 dpm/nmol) and 50 μg of LDL or cyclohexanedione-LDL per ml (middle column). In control experiments, no granules were added (right column). After incubation at 37°C for 18 hr, the cellular content of cholesteryl[$^{14}$C]oleate was determined. Each value is the mean of duplicate incubations. Numbers in parentheses represent percentages of values obtained with LDL.
*The absolute binding value obtained by incubating the granules with $^{125}$I-LDL in the absence of unlabeled lipoproteins was 127 ng/μg of granule protein, and the values in the presence of unlabeled LDL or cyclohexanedione-LDL were 6.4 and 98 ng/μg of granule protein, respectively.

macrophages. In control experiments we showed that pretreatment of the granules with avidin or protamine chloride did not influence their phagocytosis (right column).

The effects of cyclohexanedione treatment of LDL on the ability of the granules to bind LDL and to mediate its uptake by macrophages are shown in Table 2. This shows that binding of $^{125}$I-LDL to granules was inhibited almost completely (95% inhibition) in the presence of excess unlabeled LDL but only partially (23% inhibition) in the presence of excess cyclohexanedione-LDL, reflecting the greatly reduced ability of cyclohexanedione-LDL to bind to the granules (left column). When cyclohexanedione-LDL was then incubated with monolayers of mouse macrophages in the presence of mast cell granules, the granule-stimulated formation of cholesteryl esters in macrophages was only 11% of the corresponding value obtained with granules and unmodified LDL (middle column). In experiments without granules (right column) we could show that treatment of LDL with cyclohexanedione did not influence its uptake by macrophages. The above results demonstrated that binding of LDL to the granules was crucial for the granule-mediated uptake of LDL by macrophages.[†]

Under physiologic conditions, mast cell granules can be found in the extracellular space only after the mast cells have been stimulated. To investigate the relation between stimulation of mast cells and the granule-mediated uptake of LDL by macrophages, we cocultured mast cells with macrophages in the presence of LDL. Predictable amounts of granules are released from mast cells when these are stimulated with compound 48/80, an agent known to cause specific and noncytotoxic degranulation of mast cells (12). When increasing amounts of 48/80 were added to the culture medium, increasing amounts of histamine were released from the mast cells, reflecting a dose-dependent exocytosis of mast cell granules (Fig. 3A). The abilities of the various amounts of granules released to stimulate uptake of LDL by macrophages were then quantified by measuring the stimulation of cholesteryl ester synthesis in the macrophages. It appeared that the rate of cholesteryl ester synthesis in macrophages

closely followed the increase in histamine release (Fig. 3B).[‡] Because of the spontaneous degranulation of mast cells (no 48/80 added), small amounts of histamine—i.e., granules— were released into the incubation medium. Similarly, small amounts of cholesteryl esters were synthesized in the macrophages even when the cocultured mast cells were not specifically stimulated. At maximal stimulation of degranulation, the amount of histamine in the extracellular space and the rate of cholesteryl ester synthesis in the macrophages were about 5-fold higher than without stimulation. Thus, a quantitative relation could be established between the extent of mast cell stimulation and the increase in cholesteryl ester synthesis in macrophages. Addition of avidin (375 μg/ml) to the incubation medium completely prevented the stimulation of cholesteryl ester synthesis in the macrophages (Fig. 3B), evidently by inhibiting the binding of LDL to the exocytosed mast cell granules.

## DISCUSSION

The present study demonstrates that rat serosal mast cells are able to promote accumulation of LDL-derived cholesterol in mouse peritoneal macrophages *in vitro*. In this process, the secretory granules of the mast cells play an essential part. Under normal conditions these granules are located intracellularly in the mast cell cytoplasm and hence do not come in contact with the LDL in the extracellular fluid. In consequence, interaction between the granules and LDL can occur only after exocytosis of the granules, which depends on stimulation of the mast cells. In our *in vitro* model we used the compound 48/80 to stimulate the mast cells and induce their degranulation. Since, however, irrespective of the type of the stimulus, the process of degranulation of rat serosal mast cells is identical (13), any degranulating stimulus, whether immunologic or nonimmunologic, is likely to initiate the sequence of events described, which leads to uptake of LDL by macrophages.

The low-molecular-weight glycosaminoglycans, such as heparin, are able to bind LDL and form insoluble complexes with it but do not mediate its uptake by macrophages *in vitro* (14). On the other hand, aggregates of natural proteoglycans, such as those isolated from the arterial wall, which also form complexes with LDL, are able to promote the uptake of LDL

[†]In a control experiment we incubated LDL with granules in the absence of cells and then removed the granule-bound LDL by sedimenting the granules (2). When the unbound LDL present in the supernatant was then incubated with macrophages no stimulation of cholesteryl ester synthesis was observed, indicating that the granules did not modify LDL into a form that could have been taken up by macrophages in the absence of granules.

[‡]In control incubations, 48/80 alone was shown to be without effect on cholesteryl ester synthesis in macrophages. Furthermore, no cholesteryl ester synthesis was observed in the mast cells.
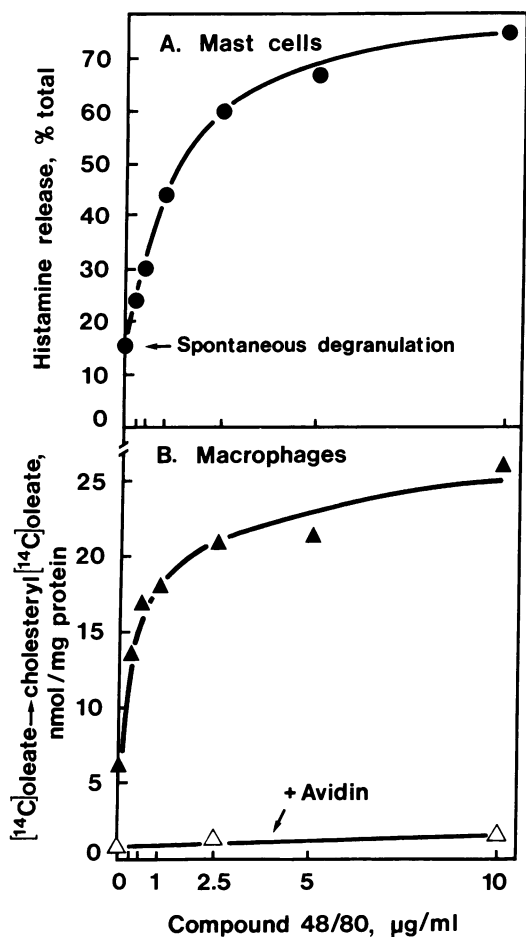
FIG. 3. Degranulation of mast cells by compound 48/80 (*A*) and concomitant stimulation of cholesteryl ester synthesis in macrophages (*B*). Each macrophage monolayer received $6.8 \times 10^5$ purified mast cells in 1 ml of medium B. After incubation at 37°C for 15 min, the mast cells were stimulated by addition of the indicated amounts of compound 48/80, and incubation was continued for 15 min to allow completion of mast cell degranulation. Finally, each dish received 50 $\mu$g of LDL and [$^{14}$C]oleate-albumin (10,300 dpm/nmol) to reach a final oleate concentration of 200 $\mu$M. To a series of dishes, 375 $\mu$g of avidin ($\triangle$) was also added. After incubation at 37°C for 18 hr, the medium was removed, the mast cells were sedimented, and the contents of histamine in the supernatants were determined. The amount of histamine released is expressed in % of the total amount of histamine contained in the mast cells. The content of cholesteryl[$^{14}$C]oleate in macrophage monolayers was determined (see text). The value of [$^{14}$C]oleate incorporated into cholesteryl esters (0.8 nmol/mg of protein) in macrophage monolayers incubated with LDL alone has been subtracted from the values obtained with LDL and mast cells.

by macrophages (15, 16). Hence, for promotion of LDL uptake by macrophages, the carrier molecules should have two properties: (*i*) an ability to bind LDL and (*ii*) an intrinsic tendency to be phagocytosed by macrophages. The heparin of rat serosal mast cells, which is a high-molecular-weight proteoglycan, has the required properties: it binds LDL (2), and it is phagocytosed by macrophages, as demonstrated in the present work. However, the heparin proteoglycan is only one of several components contained in the exocytosed granules, and from the present data it is not possible to define its exact role in the phagocytosis of the LDL–granule complexes.

A special feature of the exocytosis of mast cells is that the exocytosed granules, being large particulate cell organelles, apparently remain in the microenvironment of their parent cells (17). Hence, the granule-mediated promotion of LDL uptake by macrophages *in vivo*, if it occurs, must be a local phenomenon restricted to those tissues in which mast cells have undergone degranulation. Mast cells are present in the loose connective tissue of many organs, and their presence has also been demonstrated in the intima of the human aorta (18). Assuming that stimulation of human mast cells in the arterial intima leads to a sequence of events similar to that described here for rat serosal mast cells *in vitro*, then LDL-derived cholesterol would accumulate in arterial macrophages, a phenomenon typical of atherosclerosis.

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