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(57) **Abrégé/Abstract:**

The present invention relates to a novel drug candidate having a potential for universal therapy of allergy and asthma. The invention provides a Fab (antibody fragment), having the following characteristics: a) inhibits the IgE- FcεRI interaction; b) binds to free and cell-bound IgE; and c) is non-anaphylactic.



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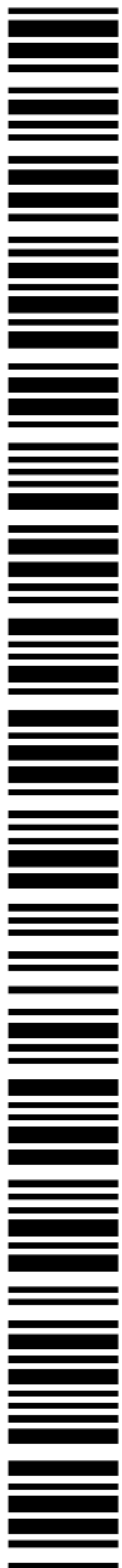
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(54) Title: NOVEL COMPOUND FOR TREATMENT OF ALLERGY AND ASTHMA

(57) Abstract: The present invention relates to a novel drug candidate having a potential for universal therapy of allergy and asthma. The invention provides a Fab (antibody fragment), having the following characteristics: a) inhibits the IgE- FcεRI interaction; b) binds to free and cell-bound IgE; and c) is non-anaphylactic.



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NOVEL COMPOUND FOR TREATMENT OF ALLERGY AND ASTHMA

Field of the invention

The present invention relates to a novel compound for treatment of allergy and asthma. More precisely, the invention relates to a novel anti-IgE Fab (antibody fragment) and medical use thereof.

Background of the invention

Type I allergy represents an immunologically-mediated hypersensitivity disease which affects almost 25% of the population worldwide [1]. Allergic patients suffer from the increased and inappropriate production of IgE antibodies against otherwise harmless antigens (pollen-, mite-, mould-, hair/dander-allergens) [1, 2]. Allergen-mediated crosslinking of IgE antibodies bound to effector cells (e. g., mast cells, basophils) *via* FcεRI induces the immediate release of biologically active mediators (histamine, leukotrienes) and causes the acute symptoms of atopy (e. g., allergic rhinitis, conjunctivitis, asthma and anaphylactic shock) [3]. When allergens are presented *via* IgE-FcεRI on professional antigen-presenting cells (e. g., monocytes, dendritic cells), T cells become activated and release Th2 cytokines thus leading to chronic, delayed disease manifestations (atopic dermatitis, chronic asthma) [4-6].

The interaction of allergens, allergen-specific IgE and FcεRI therefore represents a key pathomechanism in atopy and many forms of asthma. Since the identification and characterization of IgE antibodies [7, 8] and FcεRI [9], considerable effort has been spent in the analysis of their interactive domains and in particular to identify competitors of this interaction for a universal therapy of atopic disease [10].

Attempts to determine the interactive sites between human IgE and the alpha chain of FcεRI [11-14] comprised the screening of recombinant proteins/peptides [15-17], mutant proteins [14], chemically synthesized peptides [18, 19], and structural

analyses [20]. Furthermore, attempts were made to induce autoantibody responses against the receptor binding site of IgE [21] in order to prevent IgE binding to FcεRI. Other therapeutic approaches comprise the development of IgE-derived peptides [15, 18, 19], nucleic acids [22] and humanized anti-IgE antibodies [23-25]. Most of the described competitors result from laborious structural and rationale design [19] combined with evaluation in sophisticated cellular assays (e. g., basophil histamine release) [26] or from extensive *in vivo* testing [27]. Although many compounds have been described sofar, there still remains a need of improvements within this area.

Summary of the invention

According to the invention a unique compound has been identified, an antibody fragment called Fab 12, which is a novel inhibitor/competitor of the IgE- FcεRI interaction.

Fab12 may represent a candidate molecule for universal therapy of atopy and asthma because it can be used for the depletion of circulating IgE antibodies as well as of IgE-bearing cells. Primarily, Fab 12 is intended for use as a drug for treatment of acute or chronic atopy, especially for treatment of type I allergy and asthma.

In a first aspect, the invention provides an anti-IgE Fab of IgG₁ isotype, which:

- a) inhibits IgE- FcεRI interaction;
- b) specifically binds to free and cell-bound IgE;
- c) is non-anaphylactic; and
- d) specifically binds to FcεRI-bound IgE.

The Fab may be derived from a monoclonal or polyclonal antibody.

Alternatively, the Fab is synthetically or recombinantly produced.

In a preferred embodiment the Fab is a recombinant Fab comprising humanized framwork regions.

The hybridoma cell line producing the Mab 12 and Fab 12 of the invention has been deposited on March 27, 2002, under accession number 02032734 at the European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, SP4 OJG, UK.

26327-26

3

In a second aspect, the invention provides use of a Fab according to the invention for the production of a drug for treatment of atopic conditions. The atopic condition may be an acute or chronic atopic condition.

5 Furthermore, the invention relates to use of said Fab for depleting IgE and IgE bearing cells from the circulation. Preferably, this use is extracorporal.

In another aspect, the invention provides use of a Fab as described above for the treatment of atopic
10 conditions.

In another aspect, the invention provides the Fab as described above for use in the treatment of atopic conditions.

In another aspect, the invention provides an
15 antibody comprising the Fab as described above.

In another aspect, the invention provides a F(ab')₂ fragment of the antibody as described above.

Another use according to the invention is targeting of IgE bearing cells for therapeutical
20 intervention at the cellular level. The Fab according to the invention is then provided, for example by derivatization, with e.g. a toxin in the purpose of inactivating effector cells, plasma cells (B cells) and/or antigen presenting cells.

25 Detailed description of the invention

The invention will now be described more closely in association with an experimental section as well as the accompanying drawings:

26327-26

3a

Fig. 1 Electron micrographs. Electron micrograph of a field of unreacted chimeric Bip 1 IgE molecules. (a). Electron micrographic gallery and interpretive diagram of IgE in complex with a molar excess (b) or with an equimolar amount of mAb 12 F(ab')₂ (c). (d) shows IgE reacted with a molar excess of mAb12 F(ab')₂. An upturned C-terminal region of IgE Fc is indicated by stipples. Arrows indicate regions where the protein is thickest in the Z axis (d). The bars in all Figures correspond to 25 nm. Open figures in the diagrams represent IgE and solid figures mAb12 F(ab')₂.

Fig. 2. mAb 12 can desensitize human basophils. Enriched basophils were preincubated with either mAb 12, E-124-2-8/Dε2, a control antibody (co-ab) without reactivity to IgE or with buffer alone. Cells were then exposed to various concentrations of mAb 12 (a) or E-124-2-8/Dε2 (b) (x-axis). The percentages of released histamine in comparison with total histamine are shown on the y-axis. Results represent means of triplicates.

Fig. 3. Fab12 lacks anaphylactic activity. Human basophils were incubated with increasing concentrations of monoclonal anti-human IgE antibodies (E-124-2-8/Dε2, ab12) and Fab12 (x-axis). The percentages of released histamine in comparison with total histamine are shown on the y-axis. Results represent means of duplicate determinations.

Fig. 4-5. Giemsa stained cytopsin sample of cells purified from the blood of an atopic patient using antibody 12. Fig. 4 gives an overview of a representative cell preparation whereas Fig. 5 shows a close up of purified basophils.

EXPERIMENTAL SECTION

I. Generation and characterization of the chimeric Bet v 1-specific IgE antibody, Bip 1

In order to establish a molecular *in vitro* model for the interaction of a major allergen, its corresponding IgE, and FcεRI, we have constructed a chimeric monoclonal IgE antibody, Bip 1, with specificity for the major birch pollen allergen, Bet v 1 [28, 29].

Messenger RNA was isolated from Bip 1 hybridoma cells [29] by use of Quick Prep Micro mRNA purification kit (Amersham Pharmacia Biotech, Uppsala, Sweden). The variable immunoglobulin heavy chain region (VH) was specifically amplified by use of the Recombinant Phage Antibody System: Mouse ScFv Module (Amersham Pharmacia Biotech, Uppsala, Sweden). The human VH-4 leader sequence (L) was amplified by PCR and subsequently fused to the Bip 1 VH region by use of PCR. Sequence determination of the LVH fragment was performed on an Automated Laser Fluorescent DNA Sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden) using the Auto Read Sequencing Kit (Amersham, Pharmacia, Biotech, Uppsala, Sweden). The LVH fragment was subcloned into the *Cla* I / *Spe* I sites of an ε-expression plasmid which contains the genomic region coding for the secreted form of human IgE under the control of Ig promotor and enhancer regions. The construct was introduced into the Bip 1 hybridoma cell line by electroporation (Gene Pulser, Biorad). Stable transfectants were selected by use of geneticin in the culture media. Fusion to SP2/0 cells was performed by use of PEG. Clones producing only IgE were identified by ELISA and subcloned to generate monoclonal

cell lines. Out of three different clones secreting chimeric Bip 1 IgE, clone 859 was selected due to superior expression (11 µg/ml) of chimeric IgE. Specific and total IgE was tested, on supernatant of clone 859 grown in serum free media, in both the Pharmacia CAP System and the Uni CAP System (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

An affinity column was prepared by coupling 5 mg rBet v 1 to an AminoLink column (Pierce, Rockford, IL). Cell culture supernatant (100 ml/run) containing chimeric Bip 1 IgE antibodies (2600 kU/1 IgE) were applied to the Bet v 1 affinity column. The column was washed with PBS, and bound chimeric Bip1 IgE was eluted with 5 M MgCl₂. The Bip1 IgE-containing eluent was dialyzed against PBS at 4°C and lyophilized. Approximately 90 µg chimeric Bip 1 IgE antibody could be purified. Purified lyophilized chimeric IgE was dissolved in phosphate buffered saline. Purity, concentration and quality of chimeric Bip 1 IgE were analyzed by non-reducing SDS-PAGE and Coomassie Brilliant Blue (Imperial Chemical Industries, Ltd., Macclesfield, U.K.) staining of the gels as well as by exposure of nitrocellulose-blotted chimeric Bip 1 IgE to anti-human IgE antibodies. Chimeric Bip 1 migrated as a single band of 190 kDa with no signs of degradation and reacted with anti-human IgE antibodies (data not shown).

II. Recombinant proteins and ¹²⁵I-labeling of purified proteins

Recombinant birch pollen allergen, rBet v 1 [28] was expressed in *E. coli* and purified as described [30]. Recombinant baculovirus-expressed alpha chain of human FcεRI was purified [17]. Comparable amounts (30 µg) of purified proteins were ¹²⁵I-labeled using the chloramine-T method and purified via a Sephadex PD10 column (Pharmacia). The specific activities of the radiolabeled proteins were determined by γ-counting of equal aliquots in a γ-counter (Wallach, Turku, Finland).

III. Anti-IgE antibodies and fragments thereof

¹²⁵I-labeled anti-human IgE antibodies (RAST) were purchased from Pharmacia & Upjohn (Uppsala, Sweden). The mouse monoclonal IgG₁ anti-birch profilin antibody, 4A6 is described [31]. A panel of 25 mouse monoclonal anti-human IgE antibodies was raised by immunization of Balb/c mice with 0.5 ml intra peritoneal

injections of purified IgE (ND) Fc. The first injection 75 µg was given in CFA (Complete Freund's Adjuvans) followed by 50 µg booster injections on day 30, 33 and 34 in physiological saline. Hybridomas were produced by fusion with the SP2/0 cell line partner. Cloning of the hybridomas was done by limiting dilution. Medium from wells with single cell origin as determined by ocular inspection was tested in ELISA using a polystyrene 96 well format with passively adsorbed IgE (ND). Affinity-purified rabbit anti-mouse IgG (Fc) was used as detecting antibody. Clonality and mouse IgG1 isotype was determined by isoelectric focusing, Phast System (Pharmacia Biotech, Uppsala, Sweden) and BIAcore measurements (Biacore, Uppsala, Sweden). mAb 12 was produced in roller bottles in DMEM in serum free media. The resulting mAb 12 containing 0.45 µm-filtered medium (Millipore, Molsheim, France) was purified using FPLC System over a 10 cm XK50 protein A Sepharose 4FF column (Pharmacia Biotech, Uppsala, Sweden) with a linear flow rate of 30 cm/h and eluted with 0.1 M citrate buffer pH=5.0. The resulting mAb containing peak was neutralised by 1.0 M NaOH and concentrated in an Amicon ultrafiltration cell with a PM 30 filter to a final concentration of 5 mg/ml followed by size exclusion chromatography on a 100 cm, XK50 Superdex 200 pg column (Pharmacia Biotech, Uppsala, Sweden) equilibrated in 0.02 M phosphate buffered saline, pH=7.4. Purity was determined to exceed 95% according to SDS-PAGE, Phast System (Pharmacia Biotech, Uppsala, Sweden). Fab12 was produced from purified mAb12 by papain digestion using an ImmunoPure Fab preparation kit (Pierce, Rockford, IL). The purity and molecular weight of the Fab12 preparation was checked by SDS-PAGE.

IV. Screening for anti-IgE antibodies

Screening for monoclonal anti-human IgE antibodies that can inhibit the interaction of chimeric Bip 1 and alpha chain was performed by overlay competition experiments. A panel of 25 mouse monoclonal anti-human IgE antibodies and, for control purposes, an isotype matched monoclonal antibody, 4A6 [31], without specificity for human IgE, purified recombinant alpha chain or buffer alone were tested for their capacity to inhibit binding of chimeric Bip 1 IgE to nitrocellulose-bound alpha chain. Chimeric Bip 1 IgE was preabsorbed with 50 µg/ml of anti-human IgE antibodies and control reagents for 4 hours at 4°C and was then

exposed to nitrocellulose-dotted recombinant alpha chain at 4°C overnight. Membranes were washed and bound chimeric Bip 1 IgE was detected with ^{125}I -labeled rBet v 1 by overnight incubation at room temperature.

One of the blocking antibodies obtained, mAb 12, as well as mAb12-derived Fab fragments exhibited the unique property to strongly inhibit the IgE-FcεRI interaction and to recognize α-chain- as well as basophil-bound IgE antibodies.

V. Immunoelectron microscopy

Immunoelectron microscopic analysis of monoclonal antibodies and immune complexes were performed by negative staining as previously described [32, 33]. IgE was viewed alone and in complex with whole or mAb 12 F(ab')₂ fragments. mAb 12 was digested with pepsin-agarose (Pierce) overnight at 37°C according to the manufacturers instructions. The F(ab')₂ fraction was separated from other digestion products by HPLC. Reactants (at ~ 1 mg/ml each) were mixed in borate buffered saline and incubated at room temperature for 30 minutes. Following incubation, the reactants were allowed to bind to carbon membranes, stained with uranyl formate, and mounted on copper grids for analysis. Electron micrographs were recorded at 100,000 fold magnification on a JEOL CX 1200 electron microscope.

Many of the uncomplexed IgE molecules did not display the clear three armed "Y"-shaped configuration typical of other monomeric Ig forms (Fig 1a). This is consistent with a recent report in which the Fc of Fab-tagged IgE showed similar configurations [34]. When mixed with whole mAb 12, a variety of small complexes, chains and rings were observed but were uninterpretable due to the difficulty in distinguishing between the IgG probe and the IgE target molecules (data not shown). Consequently, we digested mAb 12 with pepsin to yield F(ab')₂ fragments. When IgE was mixed with a molar excess of F(ab')₂, the majority of molecules formed cross-shaped figures with two longer pairs of arms at right angles to two or three shorter arms (Fig 1b). When mixed at molar equivalence, chains and, less frequently, rings of molecules were observed (Fig 1c). Closer examination revealed that the longer arms are composed of two segments each. We interpret these

images as showing two F(ab')₂ anti-IgE (the long arms positioned laterally in Fig 1b and the linking molecules of the chain in Fig 1c) reacting with epitopes on either side of the Fc of IgE at a position very close to the geometric center of the molecule. Based on previously published models of IgE, this would place the epitopes near the Cε2-3 juncture. Relatively few of the complexes clearly show three putative IgE arms (Figures 1b and 1c). Yet most showed one narrower arm (Fc) and what appeared to be a pair of arms (Fabs) in close contact or partially superimposed upon each other as depicted diagrammatically in Fig 1b and 1c.

VI. Combined toluidine blue/immunofluorescence staining of human basophils

Binding of monoclonal anti-human IgE antibodies to basophil-bound IgE was analyzed by a double staining technique using toluidine blue and indirect immunofluorescence as described [35]. Mononuclear cells enriched from the peripheral blood of two healthy volunteers were incubated with monoclonal anti-human IgE antibody E-124-2-8/Dε2 (Immunotech, Marseille, France), monoclonal anti-human IgE antibody 12, anti-IL-3Rα (CD123), monoclonal antibody 9F5 (PharMingen, San Diego, CA), and, for control purposes, with anti-human IgM antibody (PharMingen) or buffer alone for 30 min at 4°C. After incubation, mononuclear cells were washed twice in phosphate buffered saline, and then exposed to fluorescein-labeled goat F(ab')₂ anti-mouse IgG for 30 min at 4°C. Cells were fixed in 0.025% glutaraldehyde solution for 1 min, and incubated with toluidine blue (0.0125%) for 12 min at room temperature. After washing, cells were analyzed using a fluorescence microscope (Olympus, Vienna).

mAb 12 differs substantially from the previously described anti-human IgE antibodies (e. g., BSW17) [36, 37] as, despite its ability to block the binding of human IgE to FcεRI and its reactivity to the FcεRI-binding region of human IgE, mAb 12 was able to recognize alpha chain- as well as basophil-bound IgE antibodies. The most likely explanation for this is that mAb 12 interacts exactly with or very close to the receptor binding site of human IgE and due to the 1:1 stoichiometry of the IgE-FcεRI interaction [38, 39] binds to one of the IgE constant domains whereas the second domain can interact with the alpha chain. This assumption is supported by results obtained via two types of experiments: 1.) mAb

12-derived Fabs despite reacting with receptor-bound IgE and preventing IgE binding to the receptor did not induce histamine release from human basophils and 2.) Negative stain immunoelectron microscopy showed that mAb 12 F(ab')₂ bound on either side of the Fc of IgE near the Cε₂-3 juncture.

VII. Histamine release experiments

Histamine release experiments were performed with peripheral blood polymorphonuclear leukocytes (PMN) from healthy volunteers using anti-human IgE antibodies E-124-2-8/Dε₂ (Immunotech, Marseille, France), mAb 12, as well as with Fab12. PMN were enriched from heparinized blood samples using Dextran T70 [40], washed and resuspended in histamine release buffer (HRB). HRB containing 25 mM Tris (pH=7.6), 5 mM KCl, 130 mM NaCl, and human serum albumin at 0.33 mg/ml was used for the washing of the cells. The same buffer supplemented with 0.6 mM Ca²⁺ and 1mM Mg²⁺ was used in histamine release experiments. The capacity of anti-human IgE antibodies to induce basophil histamine release by crosslinking of FcεRI-bound IgE antibodies was tested by exposing PMN to various concentrations of anti-human IgE antibodies at 37°C for 30 min and measurement of liberated histamine as described [40]. The desensitizing effects of anti-human IgE antibodies E-124-2-8/Dε₂ and mAb 12 on anti-IgE-induced histamine release were analyzed as follows [41]. Briefly, PMN were first incubated either with i.) HRB containing 0.01 M EDTA, ii.) HRB containing 0.01 M EDTA and E-124-2-8/Dε₂ (1 μg/ml), iii.) HRB containing 0.01 M EDTA and mAb 12 (1 μg/ml), or iv.) HRB containing 0.01 M EDTA and an anti-human IgM antibody (1 μg/ml) (PharMingen) for 10 min at 37°C. Then, cells were washed and incubated with various concentrations of E-124-2-8/Dε₂ (0.01, 0.1, or 1.0 μg/ml) or mAb 12 (0.01, 0.1, or 1.0 μg/ml) diluted in HRB for 30 min at 37°C. Cells were centrifuged and the cell-free supernatants were recovered. Histamine released in the cell-free supernatants was measured by radioimmunoassay (Immunotech) and has been calculated as percentage of total histamine (cellular plus extracellular).

Preincubation of enriched human basophils with mAb 12 or E-124-2-8/Dε₂ in EDTA-containing HRB caused desensitization against subsequent IgE-dependent cell activation (Fig 2a and 2b). In particular, the subsequent exposure to either mAb 12 or E-124-2-8/Dε₂ produced a markedly reduced histamine release response

(Fig. 2a and 2b), when compared to preincubation of human basophils in HRB and EDTA alone, or to HRB and EDTA containing a control anti-human IgM antibody. The possibility that incubation of basophils with anti-human IgE antibodies in HRB containing EDTA has caused release of mediators was excluded by the lack of histamine in the culture supernatants obtained from granulocytes after the preincubation (data not shown).

When we incubated human PMN with increasing concentrations of purified monoclonal anti-human IgE antibodies (mAb 12 or E-124-2-8/Dε2) we found that mAb 12 induced much less (approximately 50% reduced) histamine release than E-124-2-8/Dε2 (Figure 3). The very same preparation of the mAb 12-derived Fab which had inhibited human IgE binding to alpha chain and which had reacted with alpha chain-bound IgE did not induce any histamine release up to a concentration of 5 µg/ml (Figure 3).

Fab12 is a non-anaphylactic competitor of the IgE-FcεRI interaction with a rather low molecular weight. If produced as recombinant Fab fragment containing humanized frame work regions it may be administered to atopic patients such as rhuMAb-E25 to complex and remove circulating IgE [23-25, 42]. Compared with the previously described anti-human IgE antibody (rhuMAb-E25) [23-25, 42], Fab12 has an as we believe important advantage: In addition to lack of anaphylactic activity, Fab12 reacts with IgE antibodies which are bound via FcεRI to basophils and thus presumably also to mast cells, eosinophils and antigen-presenting cells containing FcεRI-bound IgE. Fab12 may therefore not only be used for the depletion of IgE antibodies from the circulation but also to target effector as well as inducer cells of atopy for therapeutical intervention at the cellular level. When used for selective extracorporeal plasmapheresis, Fab12 will deplete IgE antibodies as well as IgE-bearing cells without requiring the administration into patients [43].

VIII. Human basophils purified from heparinized blood of an allergic patient

Isolation of IgE-bearing cells out of whole blood using mAb12 heparinized blood samples from allergic patients and non-atopic individuals were washed one time with PBS, 0.1% BSA and were incubated with mAb 12 (10 µg/ml) or with an

isotype-matched control antibody (10 µg/ml) for 30 minutes at room temperature under gentle agitation. Pretreated cells were collected by centrifugation at 800 x g for 15 minutes and were washed two times with PBS, 0.1% BSA to remove unbound antibodies. CELlection™ Dynabeads (Dynal, Hamburg, Germany) containing a human anti-mouse IgG attached via a DNA linker were washed according to the manufacturers instruction and were incubated with the pretreated cells for 30 minutes at 4°C by end over end rotation. The ratio of beads to target cells was at least 5:1. Target cells were attached to the tube wall by the Dynal Magnetic Particle Concentrator (Dynal) and the supernatant was discarded. Rosetted cells were washed two times with PBS, 0,1% BSA. After the last wash, rosetted cells were gently resuspended in RPMI 1640 medium with HEPES and L-Glutamine (Gibco BRL) and 1% FCS pre-warmed to 37°C. Beads were removed from cells by adding releasing buffer, containing DNase, (Dynal) for 20 minutes at room temperature. After determination of the cell count in the sample, the identity of isolated cells was determined by Giemsa staining of cytopsin preparations. Result: Antibody 12 can be used for single step purification of human basophils. To study whether antibody 12 can be used for the depletion of IgE-bearing cells, heparinized blood samples from atopic patients were incubated with antibody 12 and Dynabeads containing human anti-mouse IgG. As exemplified in Figure 4, more than 80% pure basophils could be isolated a single step purification procedure directly from the blood of atopic patient. In addition to basophils, a small percentage other IgE-bearing cells (monocytes, lymphocytes: Figure 4, and eosinophils: data not shown) were copurified. The close up in Figure 5 shows purified basophils.

IX. Reduction of IgE level in patient serum

Patient A had a total serum IgE level of 12620 kU/L and patient B 1614 kU/L as determined by CAP-measurements. 50 ml of each sersum was applied to an affinity column which contained 10 ml gel volume of antibody 12 bound to CnBr activated Sepharose 4B. In the case of patient A, the total serum IgE was reduced to 272 kU/L and in the case of patient B to 12 kU/L after application of serum to the column. Total IgE measurements wee results of duplicate determinations. These

12

results show that antibody 12 can be used to deplete more than 95% of IgE from serum/plasma of allergic patients with high IgE levels.

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26327-26

18

CLAIMS:

1. An anti-IgE Fab of IgG₁ isotype, which:
 - a) inhibits IgE- FcεRI interaction;
 - b) specifically binds to free and cell-bound IgE;
 - 5 c) is non-anaphylactic; and
 - d) specifically binds to FcεRI-bound IgE.
2. The Fab according to claim 1 derived from a monoclonal or a polyclonal antibody.
3. Use of the Fab according to claim 1 or 2 for the
10 production of a drug for the treatment of atopic conditions.
4. The use according to claim 3, wherein the atopic condition is an acute atopic condition.
5. The use according to claim 3, wherein the atopic condition is a chronic atopic condition.
- 15 6. Use of the Fab according to claim 1 or 2 for depleting IgE and IgE bearing cells from circulation.
7. The use according to claim 6, wherein the use is extracorporal.
8. An antibody comprising the Fab according to
20 claim 1 or 2.
9. A F(ab')₂ fragment of the antibody according to claim 8.
10. Use of the Fab according to claim 1 or 2 for the treatment of atopic conditions.

26327-26

19

11. The use according to claim 10, wherein the atopic condition is an acute atopic condition.

12. The use according to claim 10, wherein the atopic condition is a chronic atopic condition.

5 13. The Fab according to claim 1 or 2 for use in the treatment of atopic conditions.

14. The Fab of claim 13 for use in the treatment of an acute atopic condition.

15. The Fab of claim 13 for use in the treatment of a
10 chronic atopic condition.

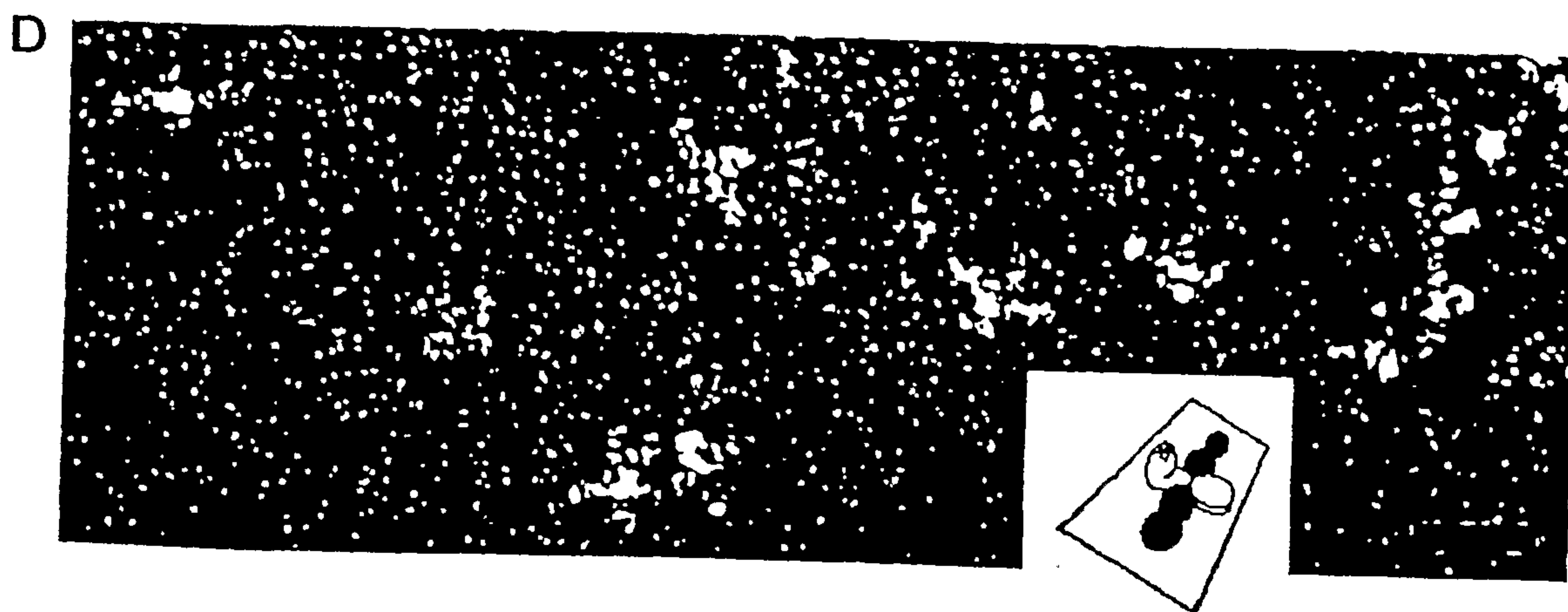
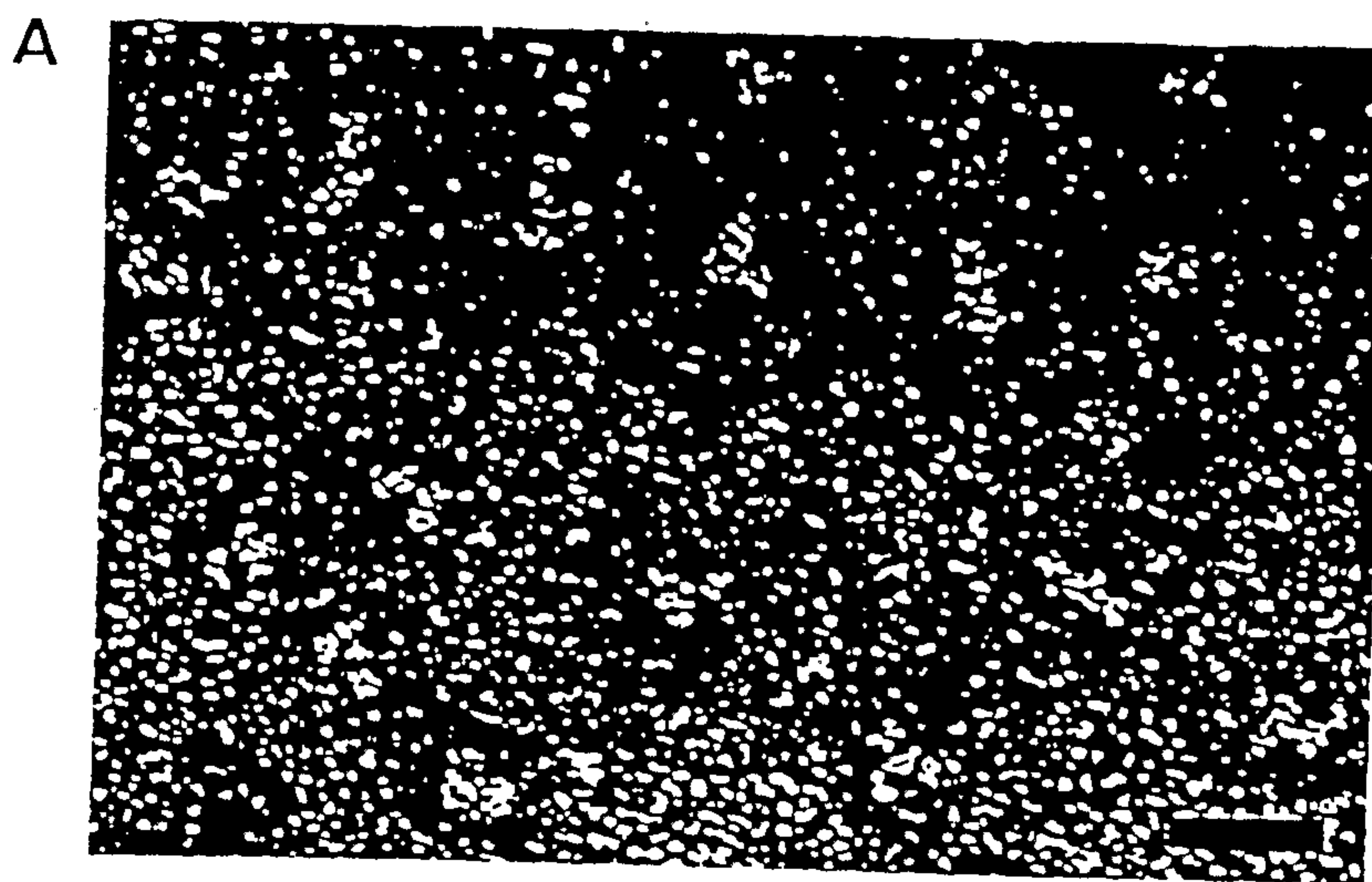
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PATENT AGENTS

1/6

FIG. 1



2/6

FIG. 1 CONT.

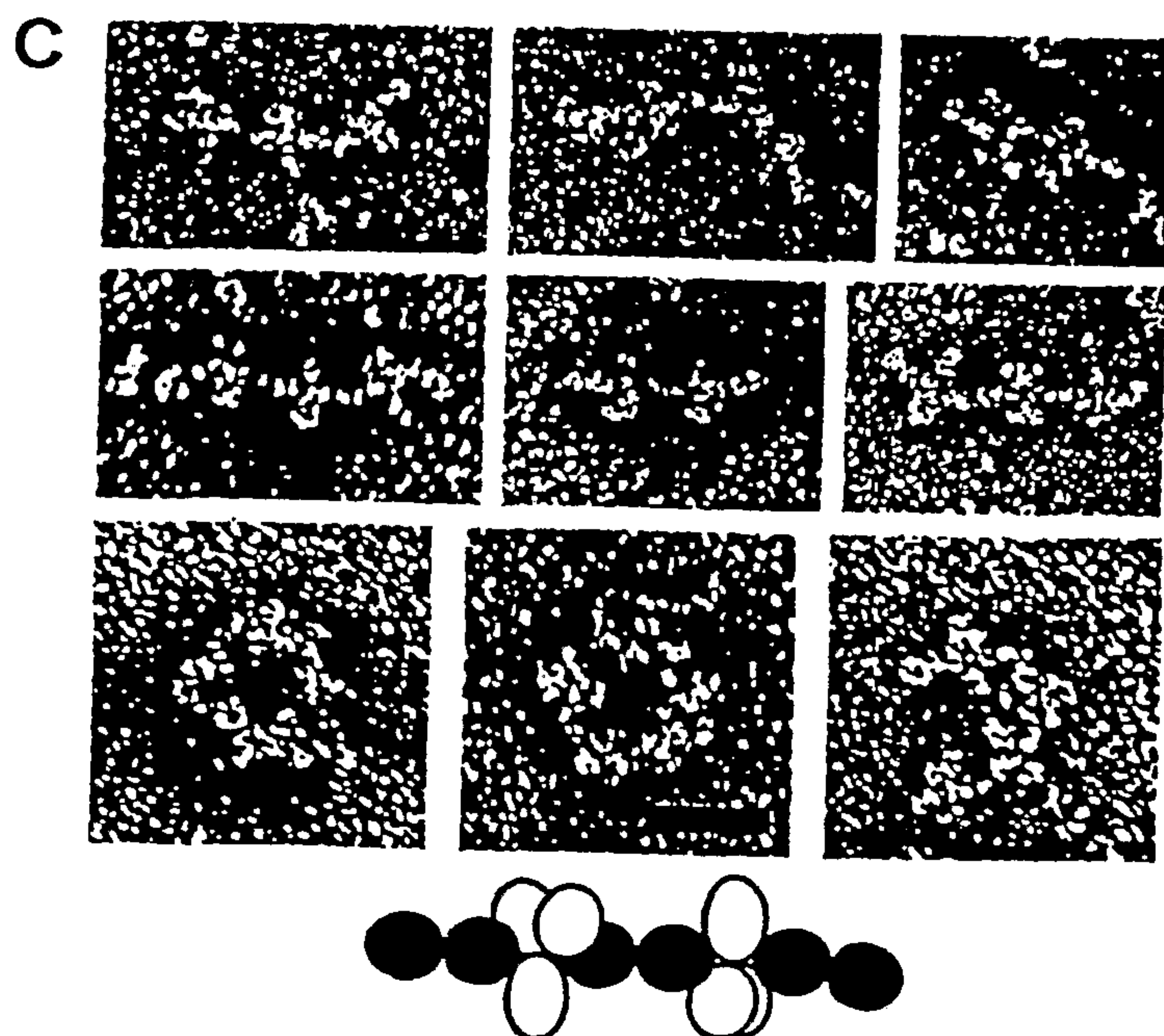
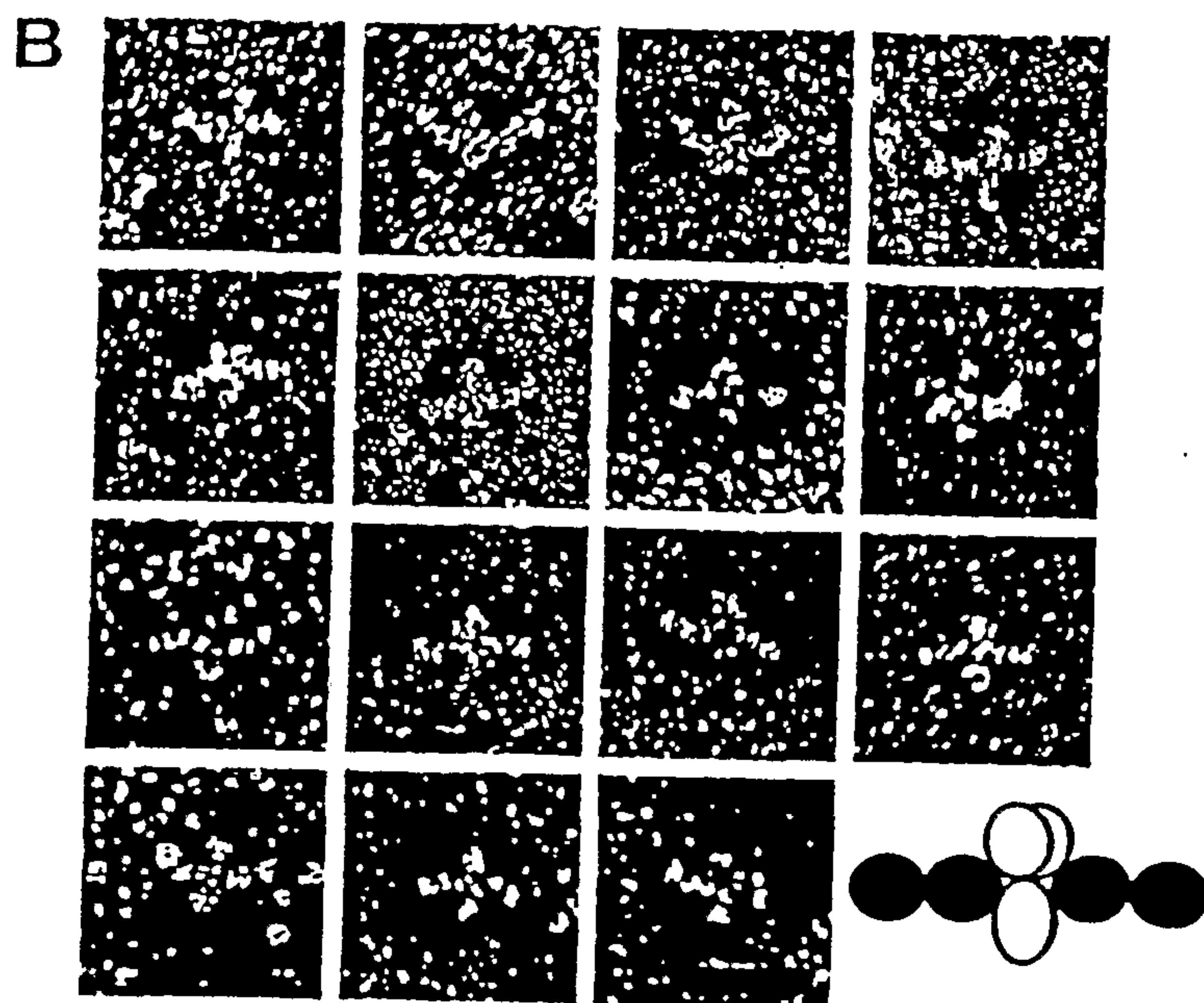
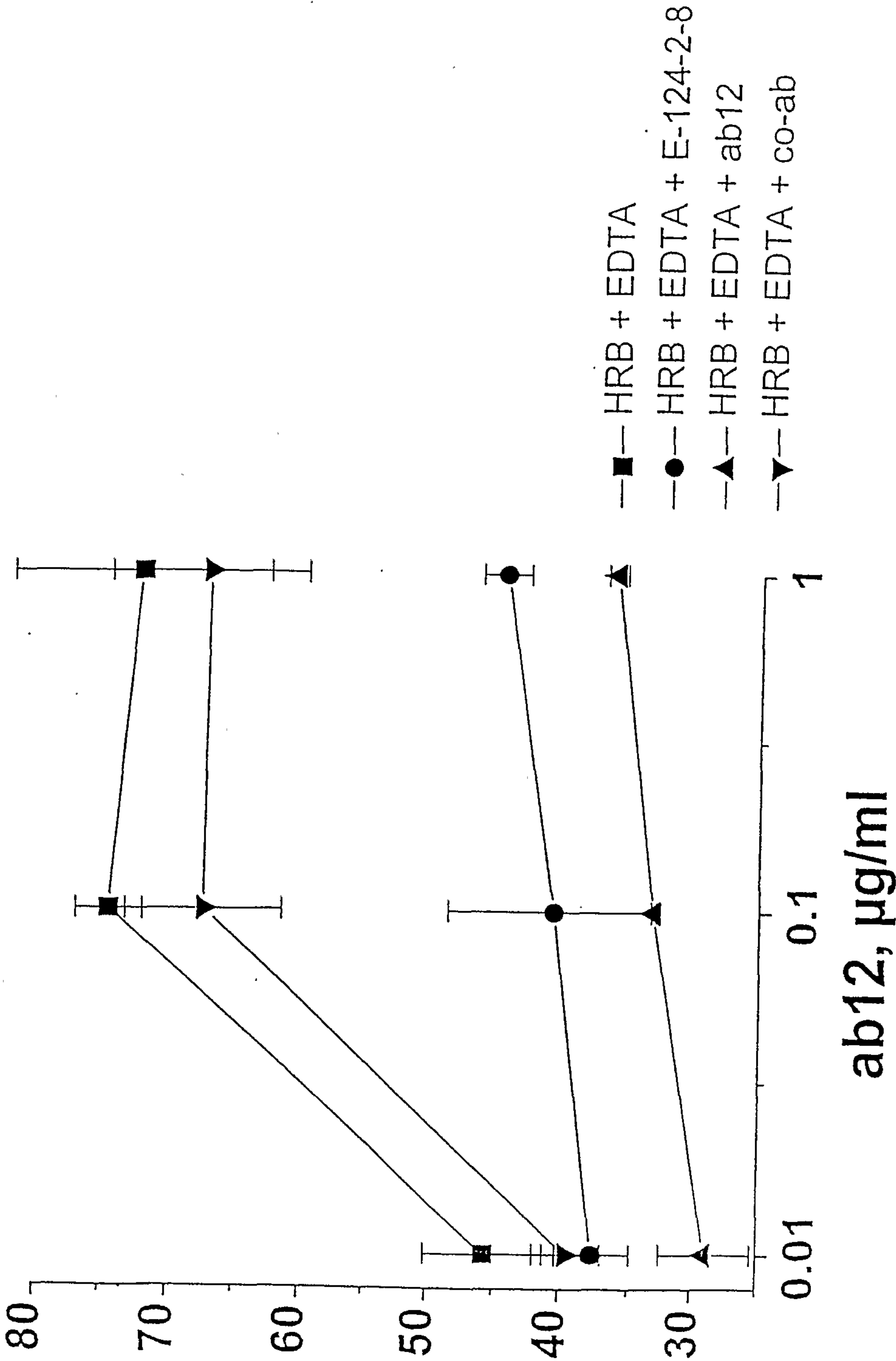
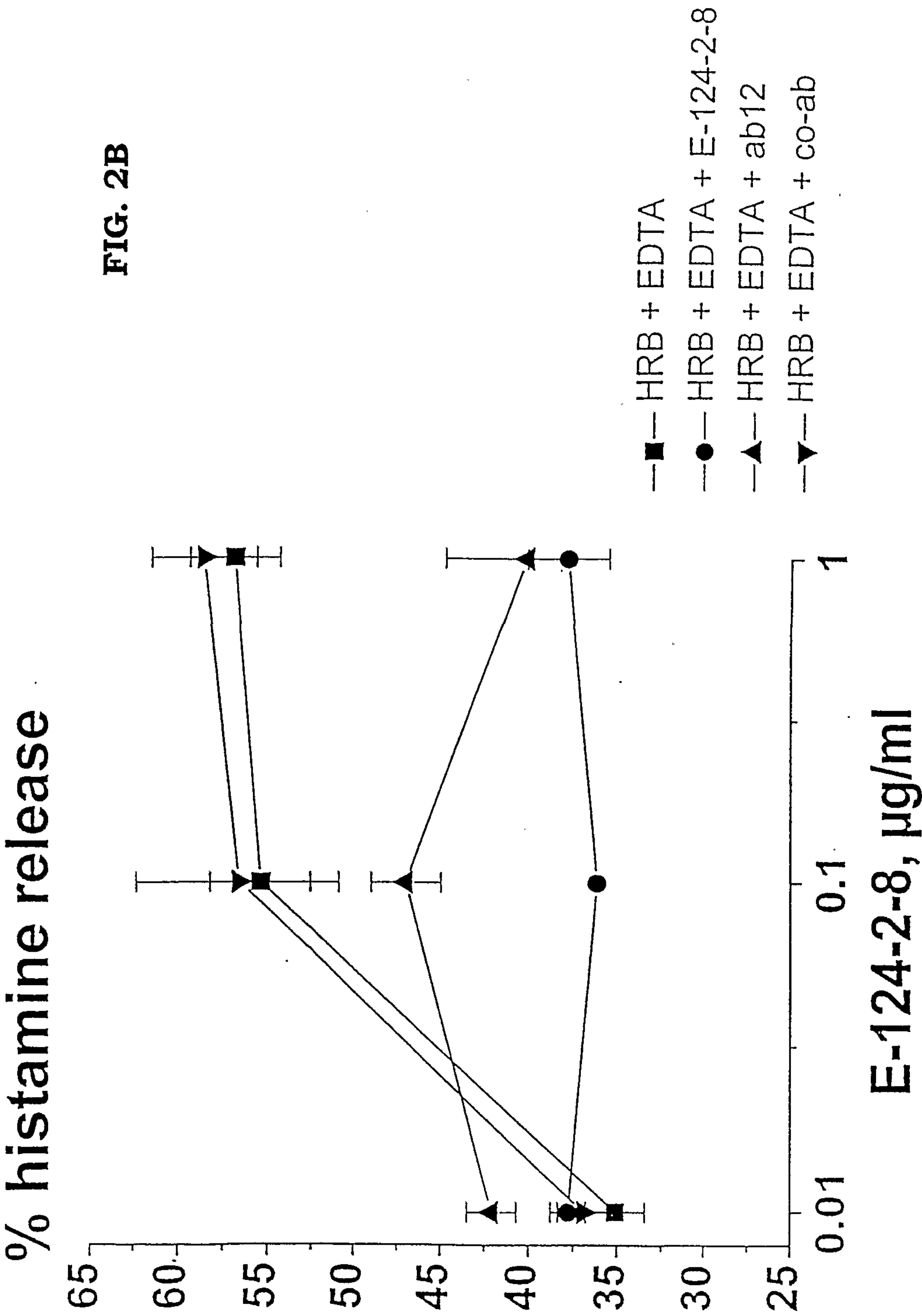


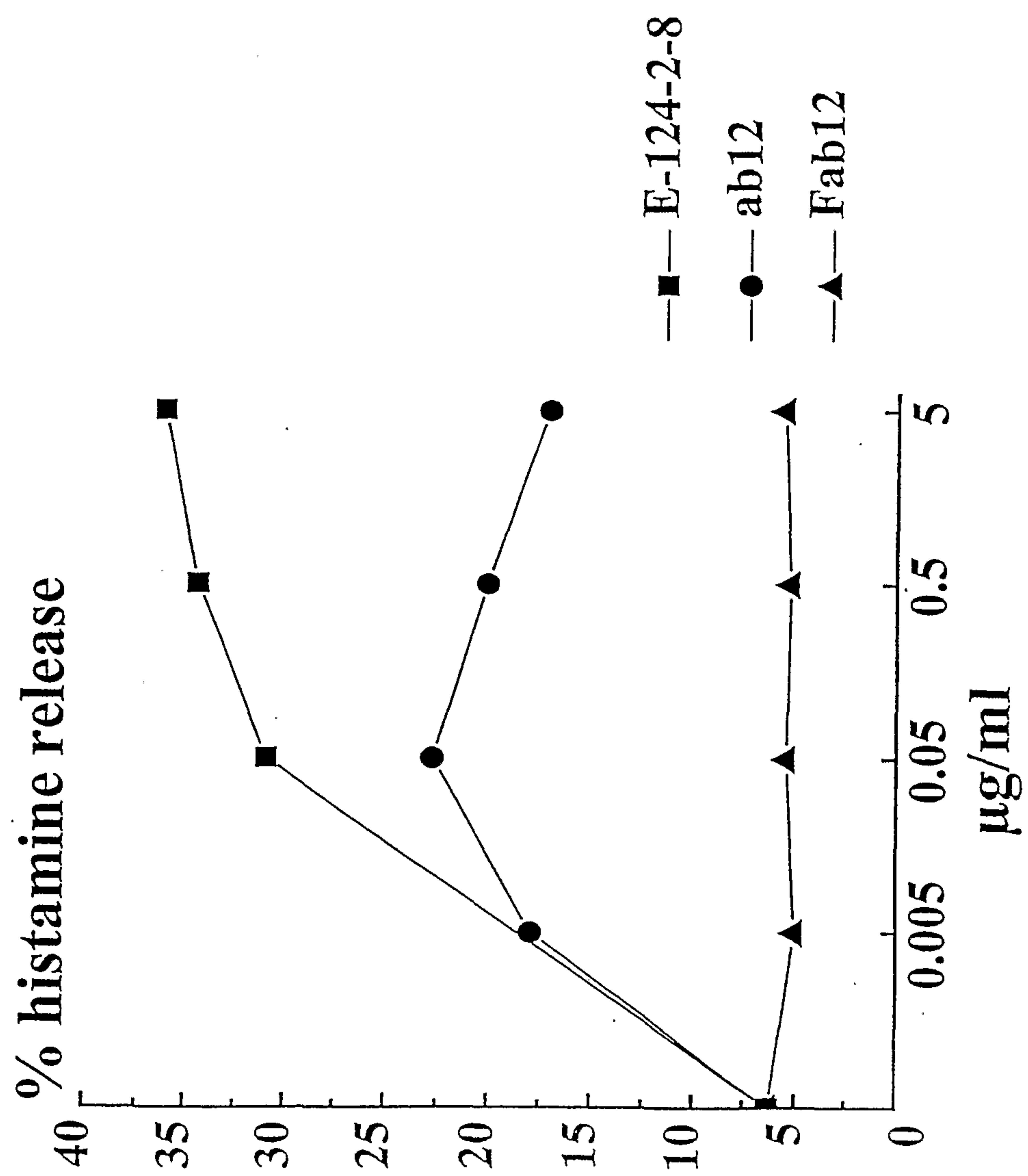
FIG. 2A





5/6

FIG. 3



6/6

FIG. 4

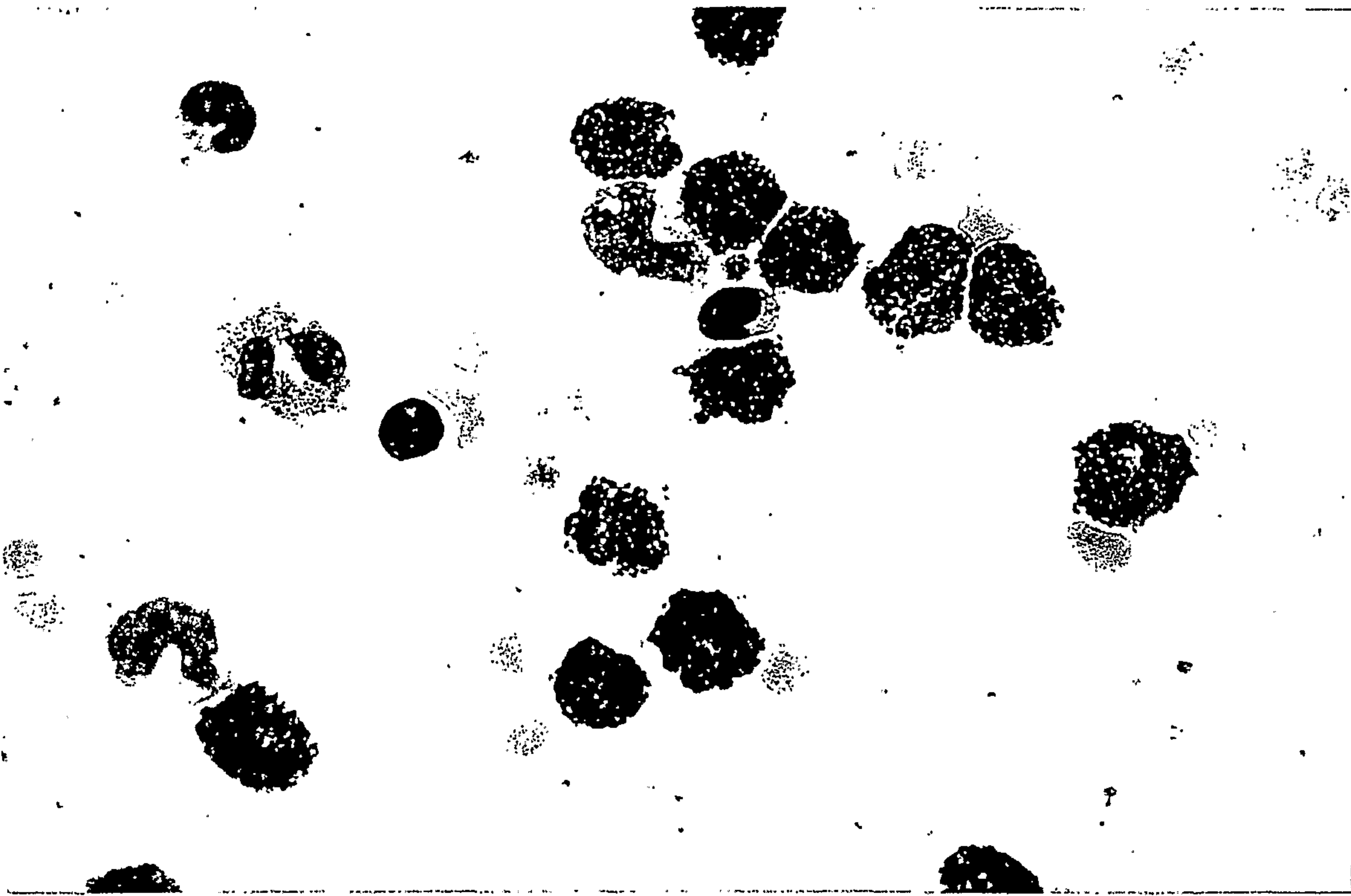


FIG. 5

