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(54) Titre : PROCEDE POUR DETERMINER DES BIOMARQUEURS LIES AU SYNDROME DE DETRESSE
RESPIRATOIRE AIGUE (SDRA), PROCEDE POUR SUIVRE LE DEVELOPPEMENT ET LE TRAITEMENT DU
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(54) Title: A METHOD FOR DETERMINING ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) RELATED
BIOMARKERS, A METHOD TO MONITOR THE DEVELOPMENT AND TREATMENT OF ARDS IN A PATIENT

(57) **Abrégé/Abstract:**

This invention concerns methods for monitoring the development of and for treatment of ARDS in a patient. The method for monitoring the development of ARDS is based on comparing the level or activity of the biomarkers obtained in a sample drawn at a later point of time to the levels or activities of the same biomarkers in a sample drawn at a previous point of time. A favourable change in the level or activity of a certain biomarker represents a regression of the disease (recovery of the patient), and, conversely, an adverse change in the level or activity of a certain biomarker represents a worsening of the disease. If, for example, the level or activity for one or more of the biomarkers monitored discontinues to show a favourable change or starts to show an unfavourable change, the treatment of the patient is enhanced by administering a therapeutically active agent useful in the treatment of ARDS. The invention concerns further a method for simultaneous determination of a multiple of biomarkers in a sample from a patient, wherein said biomarkers are related to ARDS. The level or the activity of the biomarkers is determined. The invention also concerns a diagnostic kit useful for carrying out the method, particularly a kit comprising a chip, such as a microarray suitable for use in biochip technology.



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(54) Title: A METHOD FOR DETERMINING ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) RELATED BIOMARKERS, A METHOD TO MONITOR THE DEVELOPMENT AND TREATMENT OF ARDS IN A PATIENT

(57) Abstract: This invention concerns methods for monitoring the development of and for treatment of ARDS in a patient. The method for monitoring the development of ARDS is based on comparing the level or activity of the biomarkers obtained in a sample drawn at a later point of time to the levels or activities of the same biomarkers in a sample drawn at a previous point of time. A favourable change in the level or activity of a certain biomarker represents a regression of the disease (recovery of the patient), and, conversely, an adverse change in the level or activity of a certain biomarker represents a worsening of the disease. If, for example, the level or activity for one or more of the biomarkers monitored discontinues to show a favourable change or starts to show an unfavourable change, the treatment of the patient is enhanced by administering a therapeutically active agent useful in the treatment of ARDS. The invention concerns further a method for simultaneous determination of a multiple of biomarkers in a sample from a patient, wherein said biomarkers are related to ARDS. The level or the activity of the biomarkers is determined. The invention also concerns a diagnostic kit useful for carrying out the method, particularly a kit comprising a chip, such as a microarray suitable for use in biochip technology.

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A METHOD FOR DETERMINING ACUTE RESPIRATORY DISTRESS
SYNDROME (ARDS) RELATED BIOMARKERS, A METHOD TO MONITOR
THE DEVELOPMENT AND TREATMENT OF ARDS IN A PATIENT

5 FIELD OF THE INVENTION

This invention concerns methods for monitoring the development of and for
treatment of acute respiratory distress syndrome (ARDS) in a patient. The method
for monitoring the development of ARDS is based on comparing the level or
10 activity of the biomarkers obtained in a sample drawn at a later point of time to the
levels or activities of the same biomarkers in a sample drawn at a previous point of
time. A favourable change in the level or activity of a certain biomarker represents a
regression of the disease (recovery of the patient), and, conversely, an adverse
change in the level or activity of a certain biomarker represents a worsening of the
15 disease. If, for example, the level or activity for one or more of the biomarkers
monitored discontinues to show a favourable change or starts to show an
unfavourable change, the treatment of the patient is enhanced by administering a
therapeutically active agent useful in the treatment of ARDS.

20 The invention concerns further a method for simultaneous determination of a
multiple of biomarkers in a sample from a patient, wherein said biomarkers are
related to ARDS. The levels or the activities of the biomarkers are determined. The
invention also concerns a diagnostic kit useful for carrying out the method,
particularly a kit comprising a chip, such as a microarray suitable for use in biochip
25 technology.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the
30 invention, and in particular, cases to provide additional details respecting the
practice, are incorporated by reference.

Multiplex assays, i.e. methods for simultaneous detection or quantification of a multiple of analytes in a sample are as such well known. Such assays are assays that simultaneously measure multiple analytes in a single run. Multiplex assays can be classified based on how many analytes can be measured per assay: the amount of analytes range from a few (at least two) up a very high number. The commercial multiplex assays are typically designed for simultaneous detection of up to about 50 analytes. These methods can be used for analyses of nucleic acids and proteins, such as antibodies. Also carbohydrates and other chemical compounds can be measured.

10 The multiplex methods can be carried out in many alternative ways.

As an example can be mentioned a microarray which is a 2D array on a solid support that simultaneously assays a large number of biological analytes. In a protein microassay such as an antibody microassay different antibodies have been affixed on a solid support at separate locations in a predetermined pattern. These antibodies are used as capture molecules capable of capturing analytes (proteins) present in a sample.

As an example of a commercially available assay can be mentioned Luminex® xMAP Technology, which is a bead-based assay performed directly in a microtiter plate. Each assay contains a mixture of different microspheres (bead mix), where each bead type is defined by an individual fluorescent color tone for analyte classification and carries a specific capture reagent such as specific proteins (antibodies) on its surface. During incubation of the bead mix with the patient sample complementary reaction partner (antigens) bind to the capture antibodies on the micro-spheres. In a second incubation step the bound antigens are detected with labelled antibodies bearing a specific fluorescent marker. The amount of bound analyte (antigen) correlates directly to the fluorescent intensity of the detecting antibody allowing the quantification of analytes. The classification of the beads and the quantification of the antigens are performed with the Luminex analysis system, which is based on the technology of flow cytometry using two different lasers.

The use of multiple sequential biomarkers for diagnosis and prognosis of diseases has also been suggested.

Chirag R Parikh et al., Crit Care Med 2008 Vol. 36, No. 4 (Suppl); p S159-S165,
5 suggests the use of multiple sequential biomarkers for assessing the duration of AKI (acute kidney injury) and for predicting overall prognosis with respect to dialysis requirement and mortality. The biomarkers were NGAL (neutrophil gelatinase-associated lipocalin) and cystatin C in a plasma panel and NGAL, IL-18 (interleukin-18) and KIM-1 (kidney injury molecule-1) in a urine panel.

10

O Beran et al., Eur J Clin Microbiol Infect Dis (2009) 28: 793-799 describes sequential analysis of biomarkers such as IL-6 (interleukin-6), IL-1ra (interleukin-1 receptor antagonist), IL-1beta (interleukin-1beta), IL-8 (interleukin-8), MIP-1beta (macrophage inflammatory protein -1beta) and MCP-1 (monocyte chemoattractant
15 protein-1) and their correlation with IMD (invasive meningococcal disease) and the severity thereof.

WO 2009/053523, Faron Pharmaceuticals Oy, discloses that CD73 is a useful biomarker for monitoring the development of inflammatory diseases, in particular
20 SIRS (systemic inflammatory response syndrome), ALI (acute lung injury), ARDS and MOF (multi-organ failure) in a patient. Tissue fluid samples were drawn from the patients at different points of time and the CD73 activity in the samples was determined. An increased level of CD73 activity was found to correlate with regression of the disease.

25

So far, nobody has suggested the use of multiple sequential biomarkers for monitoring the development of ARDS in a patient. Particularly, nobody has suggested the use of a set of biomarkers consisting of or including CD73 and IL-6 for this purpose.

SUMMARY OF THE INVENTION

The aim of this invention is to provide methods and means to follow the severity of ARDS during the treatment period of this condition. Usually the ARDS patients receive the best possible intensive care but more importantly, the use of biomarkers to predict any pharmacological treatment is becoming very valuable asset to evaluate the efficacy of the treatment. One such treatment is Traumakine[®] FP-1201 (interferon beta), which has shown to reduce mortality of ARDS patients. By having valuable predictive data about the patient's condition the ICU doctor can optimize the care of the patient.

Thus, in one aspect, the invention concerns a method for simultaneous determination of a multiple of ARDS related biomarkers in a sample drawn from a patient, wherein one of the biomarkers is CD73 protein. According to the invention, said method comprises the steps of

- i) quantifying the levels of the biomarkers in said sample by subjecting the sample to binders recognizing the biomarkers, or
- ii) determining the activities of the biomarkers in said sample by using thin layer chromatography or by subjecting said sample to substrates for the biomarkers, and monitoring the change of said substrates.

In another aspect, this invention concerns a diagnostic kit for use in a bioaffinity assay method for simultaneous determination of a multiple of ARDS related biomarkers in a sample drawn from a patient, wherein the number of biomarkers including CD73 and additional biomarkers is at least 2, preferably 2-50, most preferably 2-8 . According to the invention said kit comprises

- a set of capture binders immobilised to a solid support or capable of being immobilised to a solid support, wherein each capture binder is specific for a certain biomarker to be determined, and
- a set of labelled bioaffinity components, wherein each such labelled bioaffinity component has a bioaffinity specific for a certain immobilised biomarker, or wherein each such labelled bioaffinity component has an ability to compete for the binding site with a certain immobilised biomarker.

In a third aspect this inventions concerns a method for monitoring the development of ARDS in a patient, wherein at least 2, preferably 2 to 50, most preferably 2 to 8 ARDS-related biomarkers in samples drawn from a patient at different points of time have been determined, and wherein one of the biomarkers is CD73 protein, said method being based on comparing the levels or activities of the biomarkers obtained in a sample drawn at a later point of time to the levels or activities of the same biomarkers in a sample drawn at a previous point of time, wherein a favourable change in the level or activity of a certain biomarker represents a regression of the disease, and wherein an adverse change in the level or activity of a certain biomarker represents a worsening of the disease.

In a fourth aspect this invention concerns a method for the treatment of a patient suffering from ARDS by administering to the patient a therapeutically active agent effective in the treatment of ARDS, wherein the administration is started as soon as one or more of the biomarkers used in monitoring of the development of ARDS according to this invention

- discontinues to show a favourable change, or
- starts to show an unfavourable change.

20

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows a support with spots capable of binding capture binders,

25 Figure 1B shows the support of Fig. 1A onto which capture binders are immobilised,

Figure 1C shows the support of Fig. 1B onto which biomarkers to be determined have been immobilised to the capture binders,

30

Figure 1D shows the support of Fig. 1C onto which labelled binders have been immobilised to the biomarkers to be determined,

Figure 2 shows a labelled antibody comprising an assembly of primary antibody directed to the biomarker and a secondary antibody, which bears the label and binds to the Fc region of the primary antibody,

- 5 Figure 3 shows a set of curves for the levels of measured biomarkers as function of time. The whole lines for B1 to B4 indicate that the changes over time are favourable. The dotted line for B5 means that the decrease of its level represents an adverse change.
- 10 Figures 4a to 4h show the level or activity of eight biomarkers as function of time for a group of patients recovering from ALI or ARDS (figures 4a-4g show the level; figure 4h show the activity).

- Figure 5 shows both the activity and level (concentration) of soluble CD73 as
- 15 function of time for one patient recovering from ALI or ARDS as an example. Soluble CD73 activity (•, left y-axis) and soluble CD73 concentration (•, right y-axis) were measured from aliquots of the same samples. Figure 5 shows that activity and concentration measurements are comparable. One can see that CD73 (figure 5) and IL-6 (figure 4b) values show a dramatic change in the plasma concentrations,
- 20 which indicate favourable changes. .

DETAILED DESCRIPTION OF THE INVENTION

- The sample can be any tissue fluid, which bathes and surrounds the cells. The term
- 25 includes, for example, blood plasma, serum, whole blood, lymph, urine, exudates (pleural, peritoneal) and cerebrospinal fluid.

- The ARDS related biomarkers refer to a set of biomarkers present in a sample derived from the patient. The set of biomarkers comprise at least two, preferably
- 30 two to eight biomarkers, particularly about five biomarkers, of which one is CD73 protein. As examples of other biomarkers can be mentioned cytokines, which are proteins or peptides used in organisms as signalling compounds. The cytokines include, for example, interferons, interleukins, particularly IL-6, chemokines such

as eotaxins. As examples of other suitable biomarkers can be mentioned CRP (C-Reactive Protein) and other pentraxins.

Preferably, the biomarkers are selected from the group including the following: IL-1•, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, Basic FGF, Eotaxin, G-CSF, GM-CSF, IFN-•, IP-10, MCP-1, MIP-1•, MIP-1•, PDGF-BB, RANTES, TNF-•, VEGF, IL-1•, IL-2R•, IL-3, IL-12 (p70), IL-16, IL-18, CTACK, GRO-•, HGF, IFN-•2, LIF, MCP-3, M-CSF, MIF, MIG, •-NGF, SCF, SCGF-•, SDF-1•, TNF-•, TRAIL, CD73, CRP, Neopterin, MxA, and beta-2-microglobulin.

A particularly preferred set of biomarkers include CD73 and IL-6, either these two biomarkers, or these two biomarkers in combination with one or a few additional biomarkers. In particular, such additional biomarker is selected from the group consisting of IL-8, IL-15, Eotaxin, MPC-1, MIP-1a and IL-1ra. Preferably, such set of biomarkers comprises three to eight of the biomarkers CD73, IL-6, IL-8, IL-15, Eotaxin, MPC-1, MIP-1a and IL-1ra, provided that at least two of them is CD73 and IL-6. A particularly preferred set comprises all the eight biomarkers CD73, IL-6, IL-8, IL-15, Eotaxin, MPC-1, MIP-1a and IL-1ra.

In one alternative, the activities of the biomarkers are determined. This is carried out, for example, either by using thin layer chromatography or by subjecting the sample to substrates for the biomarkers, and monitoring the change of said substrates.

The activity of the biomarkers can, for example, be measured using thin layer chromatography according to published protocols. The activity can be also measured using any enzymatic assay that measures the conversion of a suitable substrate. For example, for CD73 the activity can be measured by conversion of AMP or another purine mononucleotide that can be used as a CD73 substrate, into the corresponding nucleoside. For example, the assay can be based on conversion of radioactively or fluorescently labelled substrates. Detection methods can rely on the quantification of the decrease in a substrate concentration, or an increase in the

product concentration or the release of the phosphate group. The CD73 dependence of the reaction can be determined by performing the assay in the presence and absence of a known CD73 inhibitor, such as AMPCP.

- 5 Generally, reporter cell lines for activity measurements are such genetically engineered cell lines in which a compound or molecule (i.e. the biomarker) of interest specifically induces a reporter gene expression. Such reporter gene expression can result in light production or in another measurable function that is quantifiable. The quantification of the reporter gene activity can then be used to
10 calculate the activity and/or level of the compound or molecule of interest.

Monoclonal antibodies specific for the biomarkers mentioned above are described in the literature and they are available from many commercial sources such as Bio-Rad Laboratories, Inc., Jena Bioscience GmbH and Sino Biological, Inc.

15

- To qualify for an "ARDS related biomarker", said biomarker shall have been found to correlate to the status of the ARDS disease so that an altered level of the biomarker over time indicates the change of the status of the disease. Those biomarkers with strongest associations will be combined to the ARDS biomarker
20 panel. For example, an increasing level of CD73 from one point of time to a later point of time indicates the efficiency of a treatment with a therapeutically active agent and consequently, the regression of ARDS. For CRP, however, an increasing level indicates the worsening of the disease. It is therefore important to first study each suitable biomarker to found out whether an increase or decrease of its level is a
25 favourable change or an adverse change with respect to the disease status.

The "bioaffinity assay" can be either an immunoassay if the biomarkers are proteins. Alternatively, it refers to a hybridising assay if the biomarkers are nucleic acids.

- 30 The "binder" (capture binder or labelled binder) refers to antibodies or the like (for example, affibodies and aptamers) when the biomarkers to be determined are proteins or peptides. If the biomarkers are nucleic acids the binders are preferably oligonucleotides.

The term "solid support" is, for example, a microsphere or bead, which bears a label. Alternatively, the solid support refers to a microtiter plate or a chip.

Preferably, the solid support is a chip, such as a microarray, suitable for use in a
5 biochip technology. Here, the microarray is a component in a biochip technology assembly comprising further means for transduction, signal processing and displaying of the results.

The term "antibody" shall be understood to include polyclonal and monoclonal
10 antibodies, any fragment thereof and genetically engineered antibodies.

The "label" can be, for example, an enzyme or a fluorescent label. A particularly preferred group of fluorescent labels is time-resolved fluorescent labels, such as lanthanide chelates. If the label is an enzyme, a substrate for said enzyme is added,
15 wherein the subsequent reaction between the enzyme and its substrate produces a detectable signal. If the label is a fluorescent label, the excitation is carried out by radiation, for example by laser, wherein a detectable signal is created.

Preferably, all the biomarkers to be determined are proteins or peptides, each of
20 which can be immobilised to a certain capture antibody. In this case, the labelled binders are also antibodies. Each labelled antibody is directed to an epitope of a certain biomarker, where said epitope is different from that of the epitope, which binds to the capture antibody.

25 A multiple of ARDS related biomarkers are simultaneously determined from the sample using a bioaffinity assay construed for example as follows: The capture binders are immobilised onto predetermined positions on the surface of a solid support, typically onto biotinylated spots or wells of a microtiter plate or the like. The capture binders will thus become arranged in the form of an array on the solid
30 support (microtiter plate). Each capture binder is specific for a certain biomarker to be determined. Adding the sample to the array and incubating the biomarkers therein with the immobilised capture binders causes immobilising of each biomarker to the corresponding capture binder.

The detection of the biomarkers can be carried out in two ways: by a non-competitive so called "sandwich assay" or by a competitive assay. In the non-competitive assay the labelled bioaffinity components, for example labelled antibodies, are added to the plate bearing the immobilised biomarkers. After
5 incubation and optionally removal of unbound labelled bioaffinity components, the label is excited to give a detectable signal. In this kind of assays, the strength of the signal is directly proportional to the concentration of the immobilised biomarker. The position of the "sandwich" on the microtiter plate informs which biomarker has been detected.

10

In the Luminex® technology, the capture antibodies are immobilised to beads, labelled with fluorescent colours so that a certain colour refers to a certain kind of capture antibodies. Upon incubation with the sample containing the biomarkers to be detected and subsequent addition of a set of second antibodies (labelled
15 antibodies labelled with a fluorescent colour different from the bead colour) a sandwich comprising "bead - capture antibody - biomarker - labelled antibody" is formed. Each such sandwich is transported to a flow cytometer and each sandwich is classified and quantified using a dual-laser equipment.

20 In a competitive assay, the plate bearing the immobilised biomarkers derived from the sample is subjected to a set of labelled antigens, wherein each labelled antigen is capable of competing for the binding site on the capture binder with the corresponding immobilised biomarker derived from the sample. When the label is excited, the signal detected will be indirectly proportional to the concentration of
25 the biomarker derived from the sample.

The invention is illustrated more in detail by reference to the drawings in which Fig. 1A shows a support (microtiter plate) with spots S1 to S5. Capture antibodies C1 to C5 have been bound to predetermined spots (Fig. 1B), which may be biotinylated to
30 enable the binding of capture antibodies thereon. Each capture antibody is specific for a certain biomarker B1 to B5 to be determined. Upon addition of the sample to the plate shown in Fig. 1B, each capture antibody will immobilise the biomarker towards the capture antibody has been raised. Unbound biomarkers may be washed

away before the addition of labelled antibodies L1 to L5, out of which one labelled antibody is specific for a certain immobilised biomarker. Upon excitation of the label L (irradiation or addition of an enzyme substrate, depending on the label L), a detectable signal is created.

5

Although the labelled antibody can be one single antibody bearing the necessary specificity and the label, the labelled antibody can alternatively be an assembly of primary antibody (PA) directed to the biomarker and a secondary antibody (SA), which bears the label L and binds to the Fc region of the primary antibody. See Fig.

10 2. This assembly avoids the expensive process of creating labelled antibodies for every biomarker one might want to detect.

When the method is repeated with samples drawn at separate points of time, the signal obtained from each labelled antibody is registered and plotted versus time
15 (Fig. 3). For some biomarkers (B1 and B2), an increased level represents a favourable change of the patient's disease, while a decreased level of B3 and B4 also represents a favourable change. On the contrary, the decreased level of B5 represents an adverse change of the patient's disease and therefore the curve could preferably be plotted in different signs or colour so as to rapidly distinguish from
20 the curves, which represent a favourable change.

The data from the determinations are collected into a data base, optionally together with clinical observations, therapeutic measures etc.

25 The invention is illustrated by the following non-restricting examples.

EXAMPLE 1

In a clinical study 26 patients with ALI or ARDS were given doses of 10 microgram
30 of interferon beta-1a for six consecutive days. This treatment reduces the mortality by 75 % if compared to normal frequency observed without treatment. Serum samples derived from the patients were analyzed with respect to the following biomarkers: IL-1ra (ra= receptor antagonist), IL-6, IL-8, IL-15, Eotaxin, MCP-1

(monocyte chemotactic protein 1), MIP-1a (macrophage inflammatory protein) and CD73. In Figure 4, a) to h) the level of each biomarker (activity for CD73) are shown as mean value for all of the patients together with the standard errors of the means (S.E.M), is plotted versus time. Day 1 refers to the value before the
5 interferon beta administration. Day 2 refers to the value 22 hours after the first interferon beta administration; Day 3 refers to the value 22 hours after the second dose (Day 2) and so on. Day 7 represents the levels 22 hours after the last administration of interferon beta. Figure 4 shows that the level of the biomarkers IL-1ra, IL-6, IL-8, IL-15 and MCP-1 decreased rapidly with time, i.e. with recovery of
10 the patients. On the other hand, the soluble CD73 activity (nmol/mL/hr) increased until Day 9 i.e. two days after the last dose, followed by a decrease towards the baseline values. The level of the biomarkers Eotaxin and MIP-1a also increased with time, i.e. with recovery of the patients.

15 EXAMPLE 2

Soluble CD73 activity was measured from samples of one of the treated patients in the above mentioned study at indicated time points (see Figure 5) using the previously published thin layer chromatography based technique. This patient
20 showed very strong induction in the soluble CD73 activity. The soluble CD73 concentration was measured by an ELISA assay based on the use of a capture antibody and a detection antibody in a sandwich assay. The activity and concentration of the soluble CD73 were measured from aliquots of the same samples. Figure 5 shows that the soluble CD73 activity and concentration behave
25 similarly.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the expert skilled in the field that other embodiments exist and
30 do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

CLAIMS

1. A method for simultaneous determination of a multiple of ARDS (acute respiratory distress syndrome) related biomarkers in a sample drawn from a patient,
5 wherein one of the biomarkers is CD73 protein, said method comprising
 - i) quantifying the levels of the biomarkers in said sample by subjecting the sample to binders recognizing the biomarkers, or by
 - ii) determining the activities of the biomarkers in said sample by using for example thin layer chromatography or by subjecting said sample to substrates for the
10 biomarkers, and monitoring the change of said substrates.
2. The method according to claim 1, said method being a bioaffinity assay method for simultaneous determination of a multiple of ARDS related biomarkers in a sample drawn from a patient, wherein one of the biomarkers is CD73 protein, said
15 method comprising the steps of
 - a) contacting a set of capture binders with a sample comprising several biomarkers to be determined, wherein each capture binder is specific for a certain biomarker to be determined,
 - b) incubating the biomarkers in the sample with the capture binders, so that the each
20 biomarker become bound to the corresponding capture binder,
 - c) adding to the array from step b) a set of labelled bioaffinity components, wherein each such labelled bioaffinity component has a bioaffinity specific for a certain biomarker which is bound to the capture binder, or wherein each such labelled bioaffinity component has an ability to compete for the binding site on the capture
25 binder with a certain biomarker to be determined,
 - d) exciting the label so as to form a detectable signal,
 - e) comparing the signal with a control to indicate the presence of a certain biomarker and/or to indicate the level of certain biomarker in the sample.
- 30 3. The method according to claim 2 wherein
 - a) each capture binder is immobilised on a predetermined position on the surface of a support so that the capture binders become arranged in the form of an array on the support,

- b) the sample is added to the array and the biomarkers therein are incubated with the immobilised binders,
- c) a set of labelled bioaffinity components is added to the array from step b), wherein each such labelled bioaffinity component has a bioaffinity specific for a certain immobilised biomarker, or wherein each such labelled bioaffinity component has an ability to compete for the binding site on the capture binder with a certain immobilised biomarker,
- d) the label is excited so as to form a detectable signal,
- e) the signal is compared with a control to indicate the presence of a certain biomarker and/or to indicate the level of certain biomarker in the sample.
4. The method according to claim 1, 2 or 3, wherein the biomarkers in addition to CD73 are selected from the group of cytokines, particularly interleukins such as IL-6, chemokines and eotaxins, C-Reactive Protein, and other pentraxins, and wherein the number of the biomarkers, including CD73 and additional biomarkers, is at least 2, preferably 2 to 50, more preferably 2-8.
5. The method according to any of the claims 2-4, wherein the method is an immunometric method, wherein the capture binders are antibodies, aptamers or affibodies, preferably monoclonal antibodies.
6. The method according to claim 5, wherein the method is a sandwich immunoassay, wherein the labelled bioaffinity components are antibodies, aptamers or affibodies, preferably monoclonal antibodies, each directed to an other epitope on the biomarker than that occupied by the capture binder.
7. The method according to claim 5, wherein the method is a competitive method, wherein the labelled bioaffinity components are a set of labelled antigens, wherein each labelled antigen is capable of competing for the binding site on the capture binder with the corresponding immobilised biomarker derived from the sample.
8. The method according to any of the claims 2-7, wherein the label is an enzyme or a fluorescent label.

9. The method according to claim 8, wherein the label is an enzyme and a substrate for said enzyme is added, wherein the subsequent reaction between the enzyme and its substrate produces a detectable signal.

5

10. The method according to any of the preceding claims, wherein said method is repeated on another sample drawn from the patient after a certain time, and that the signals attributed to each biomarker are compared to the corresponding signals of the previous assay and that the differences are registered.

10

11. A diagnostic kit for use in a bioaffinity assay method for simultaneous determination of a multiple of ARDS related biomarkers in a sample drawn from a patient, wherein the number of biomarkers including CD73 and additional biomarkers is at least 2, preferably 2-50, most preferably 2-8, said kit comprising

15

- a set of capture binders immobilised to a solid support or capable of being immobilised to a solid support, wherein each capture binder is specific for a certain biomarker to be determined, and

- a set of labelled bioaffinity components, wherein each such labelled bioaffinity component has a bioaffinity specific for a certain immobilised biomarker, or

20

wherein each such labelled bioaffinity component has an ability to compete for the binding site with a certain immobilised biomarker.

12. The kit according to claim 11, wherein the capture binders are antibodies, preferably monoclonal antibodies.

25

13. The kit according to claim 11 or 12, wherein the labelled bioaffinity components are antibodies, preferably monoclonal antibodies, each antibody being directed to an other epitope on the biomarker than that to become occupied by the capture binder.

30

14. The kit according to any of the claims 11-13, wherein the label is an enzyme or a fluorescent label.

15. The kit according to claim 14, wherein the label is an enzyme, and wherein the kit optionally also comprises a substance containing a substrate for said enzyme.

5 16. The kit according to any one of the claims 11-15, wherein the capture binders are immobilised to a solid support which is a chip, such as a microarray, suitable for use in a biochip technology.

10 17. The kit according to claim 16, wherein said microarray is a component in a biochip technology assembly comprising further means for transduction, signal processing and displaying of the results.

18. The kit according to any one of the claims 11-15, wherein the capture binders are immobilised to a solid support which is a microsphere bearing a label.

15 19. The kit according to any one of the claims 11- 18, wherein the capture binders are biotinylated and thereby capable of being immobilised to a streptavidin-treated solid support.

20 20. A method for monitoring the development of ARDS in a patient, wherein at least 2, preferably 2-50, most preferably 2-8, ARDS-related biomarkers in samples drawn from a patient at different points of time have been determined, and wherein one of the biomarkers is CD73 protein, said method being based on comparing the levels or activities of the biomarkers obtained in a sample drawn at a later point of time to the levels or activities of the same biomarkers in a sample drawn at a
25 previous point of time, wherein a favourable change in the level or activity of a certain biomarker represents a regression of the disease, and wherein an adverse change in the level or activity of a certain biomarker represents a worsening of the disease.

30 21. The method according to claim 20, wherein the biomarkers are determined by a method according to any of the claims 1-10.

22. A method for the treatment of a patient suffering from ARDS by administering to the patient a therapeutically active agent effective in the treatment of ARDS, wherein the administration is started as soon as one or more of the biomarkers used in monitoring of the development of ARDS according to claims 20-21

- 5 - discontinues to show a favourable change, or
 - starts to show an unfavourable change.

23. The method according to claim 22 wherein the therapeutically active agent is interferon beta.

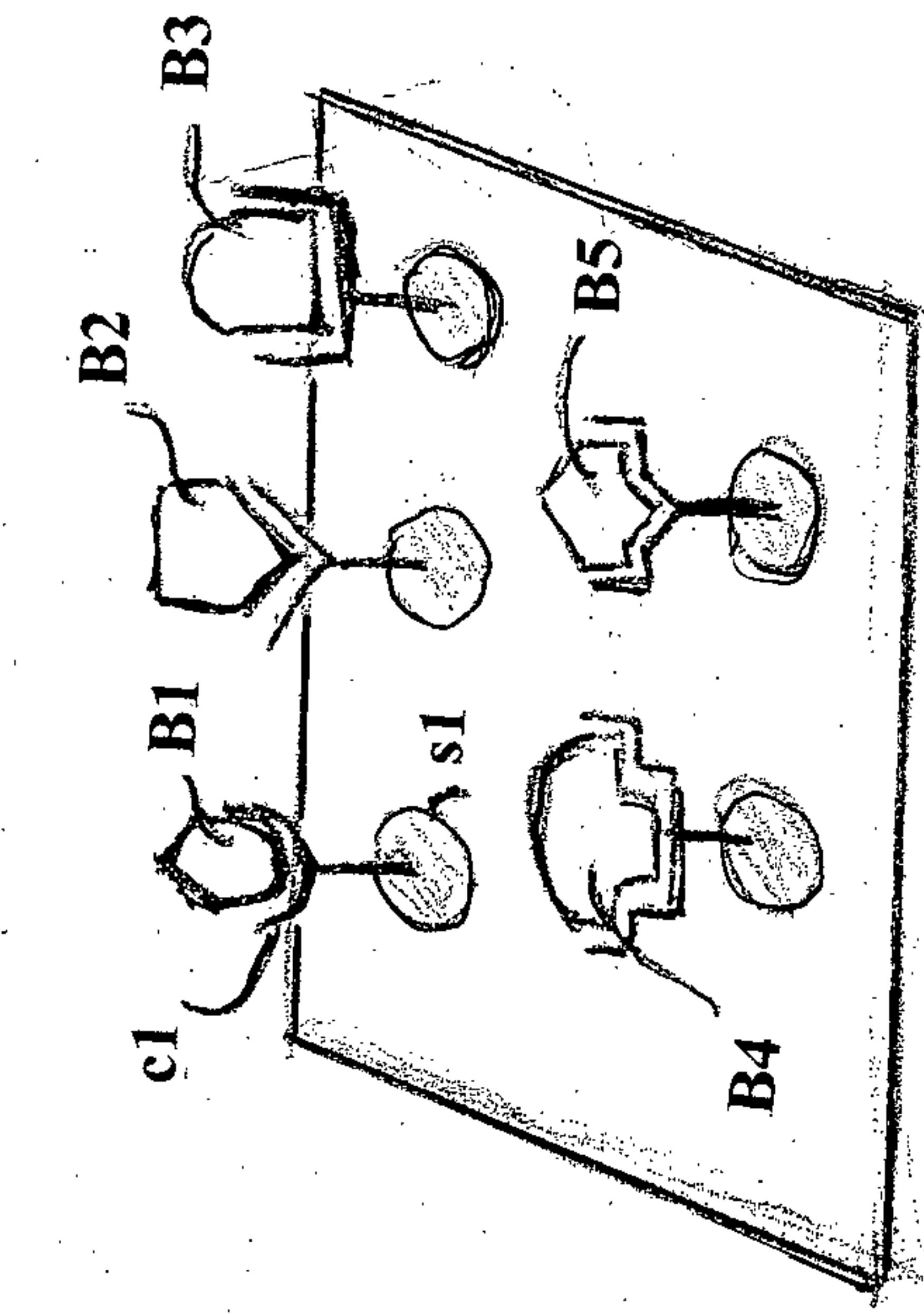


FIG. 1A

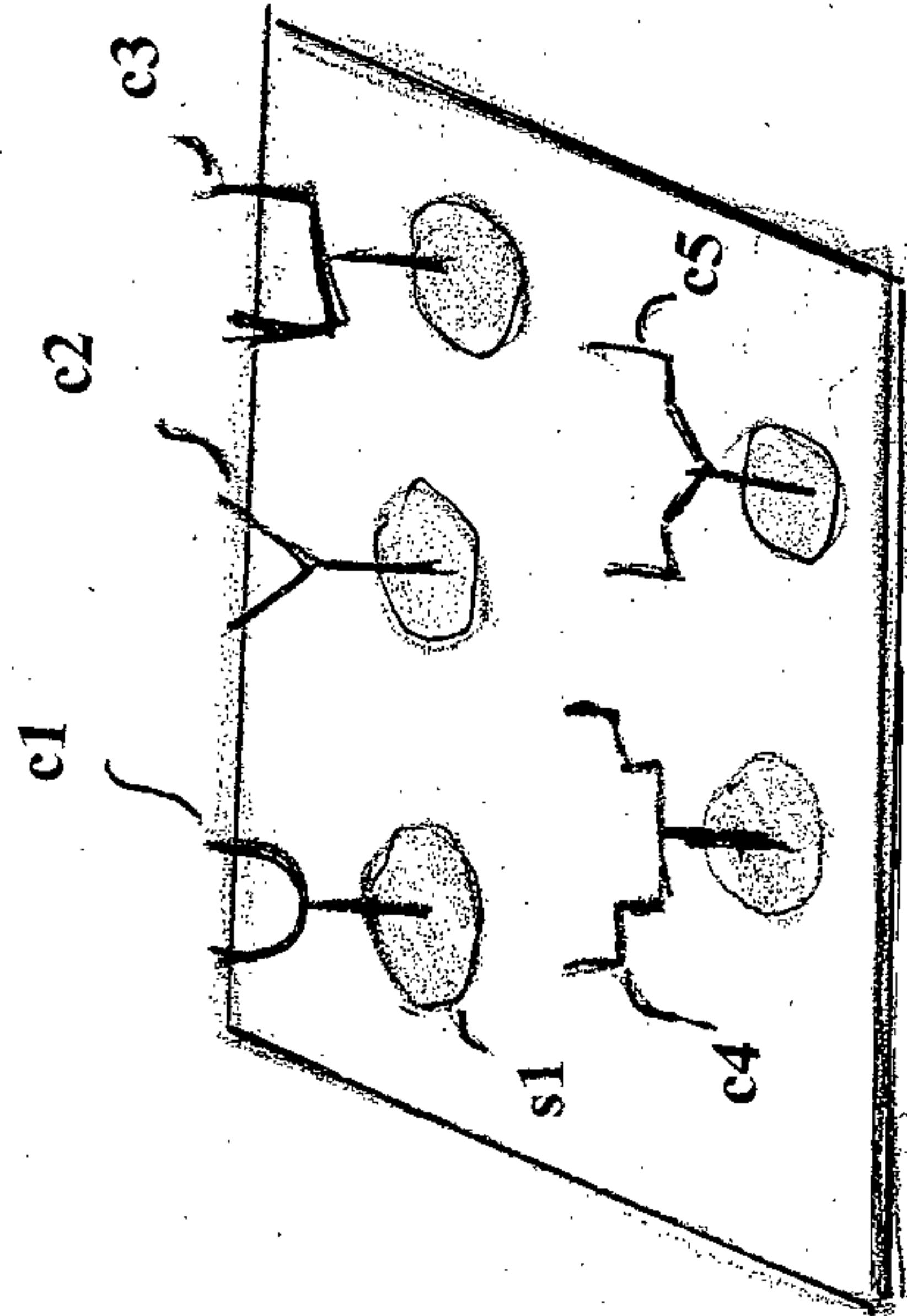


FIG. 1B

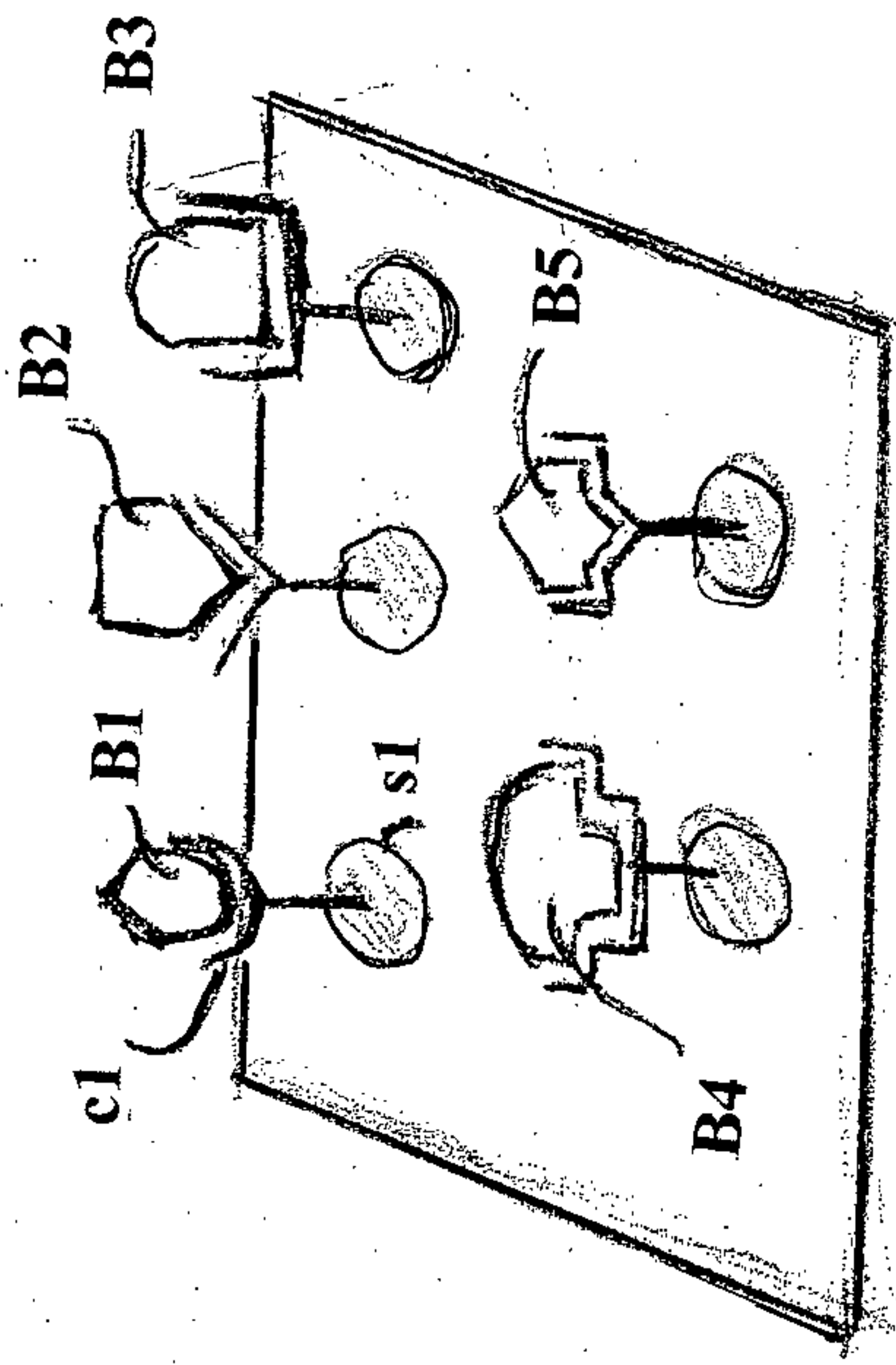


FIG. 1C

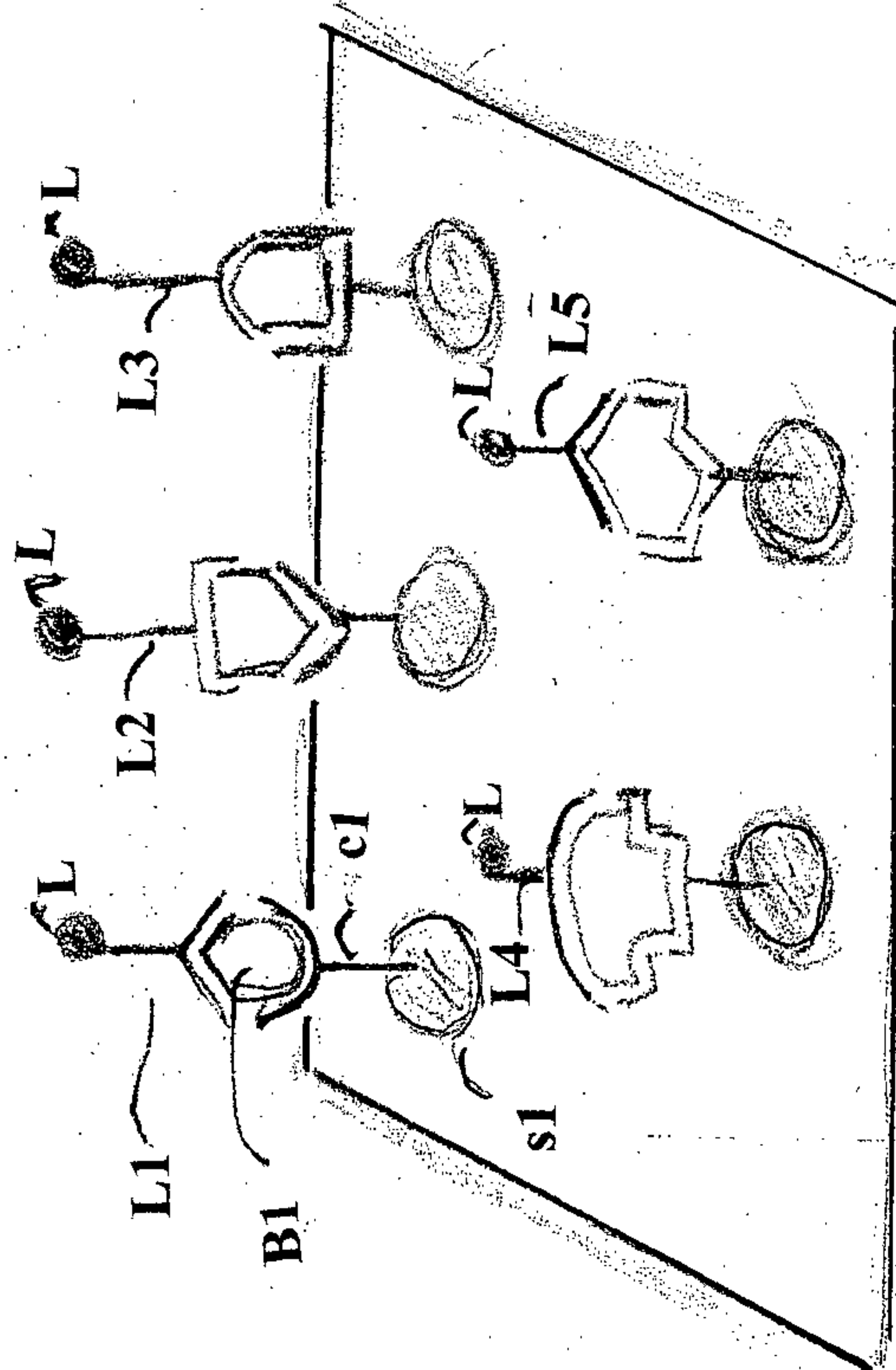


FIG. 1D

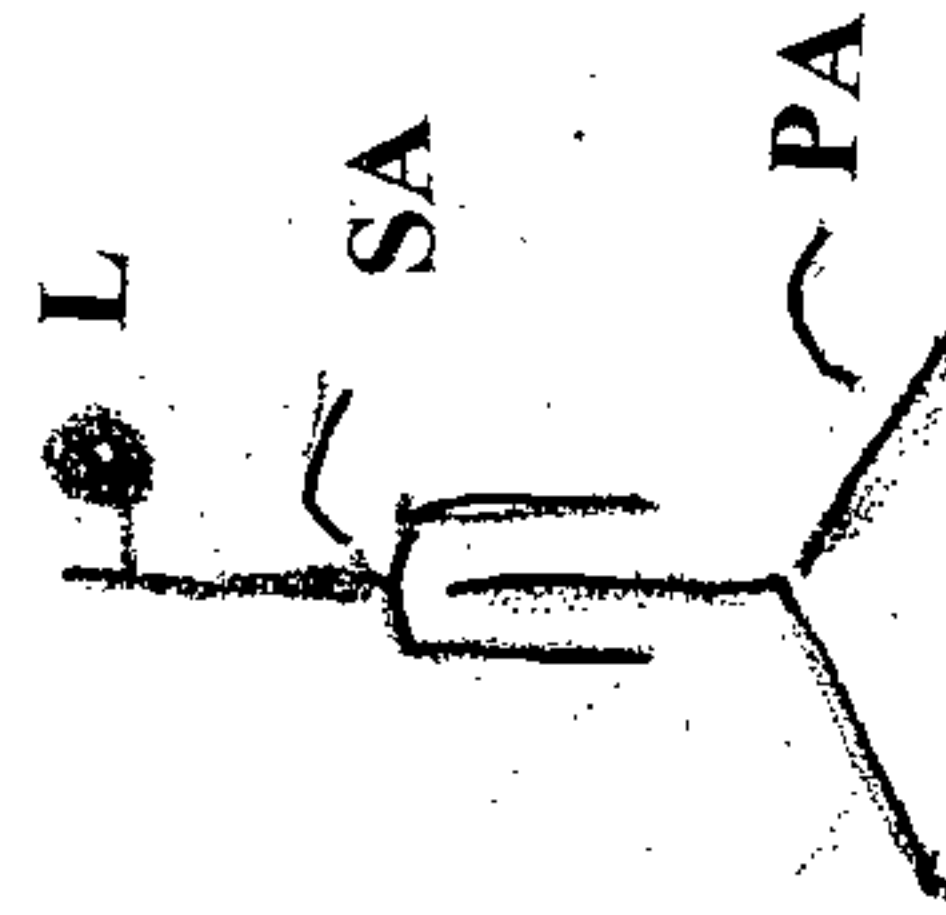


FIG. 2

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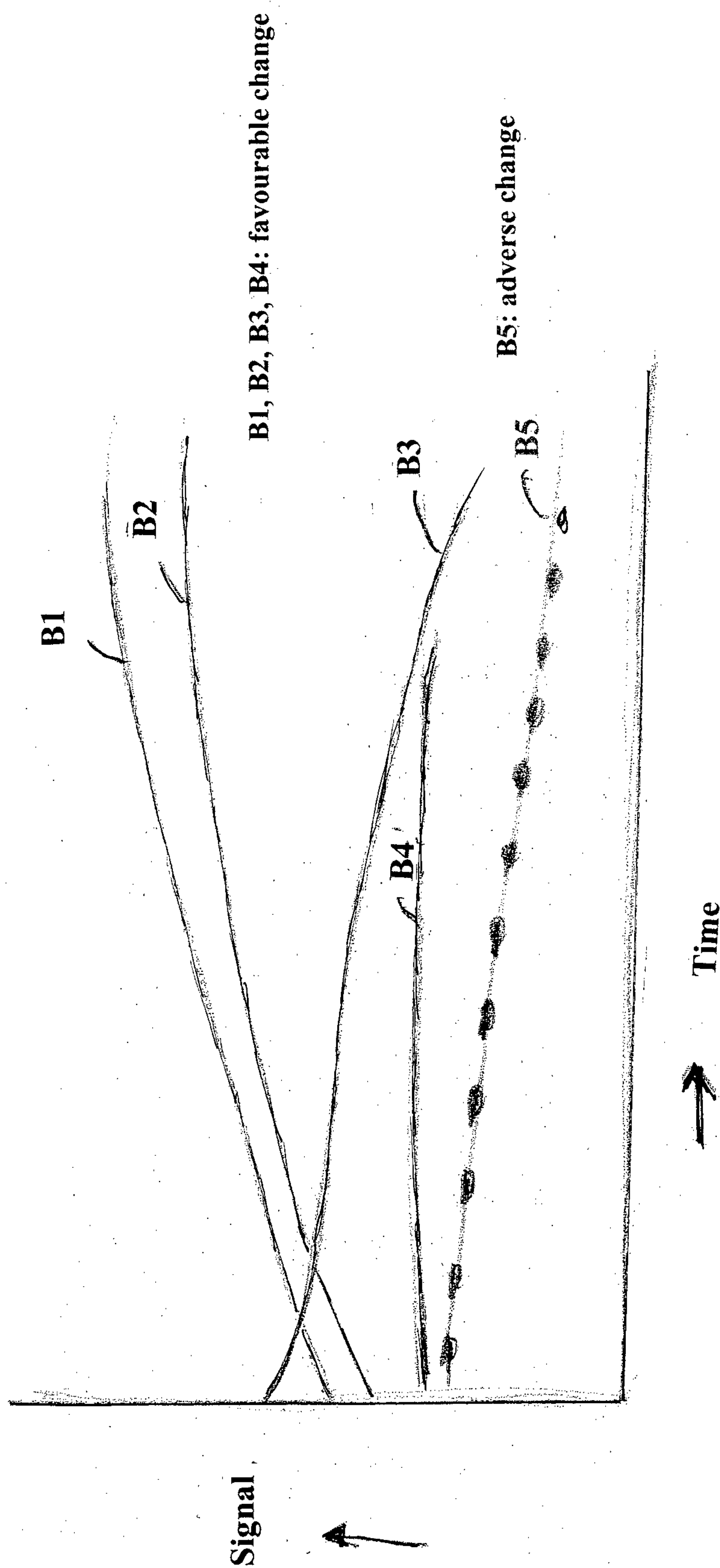


FIG. 3

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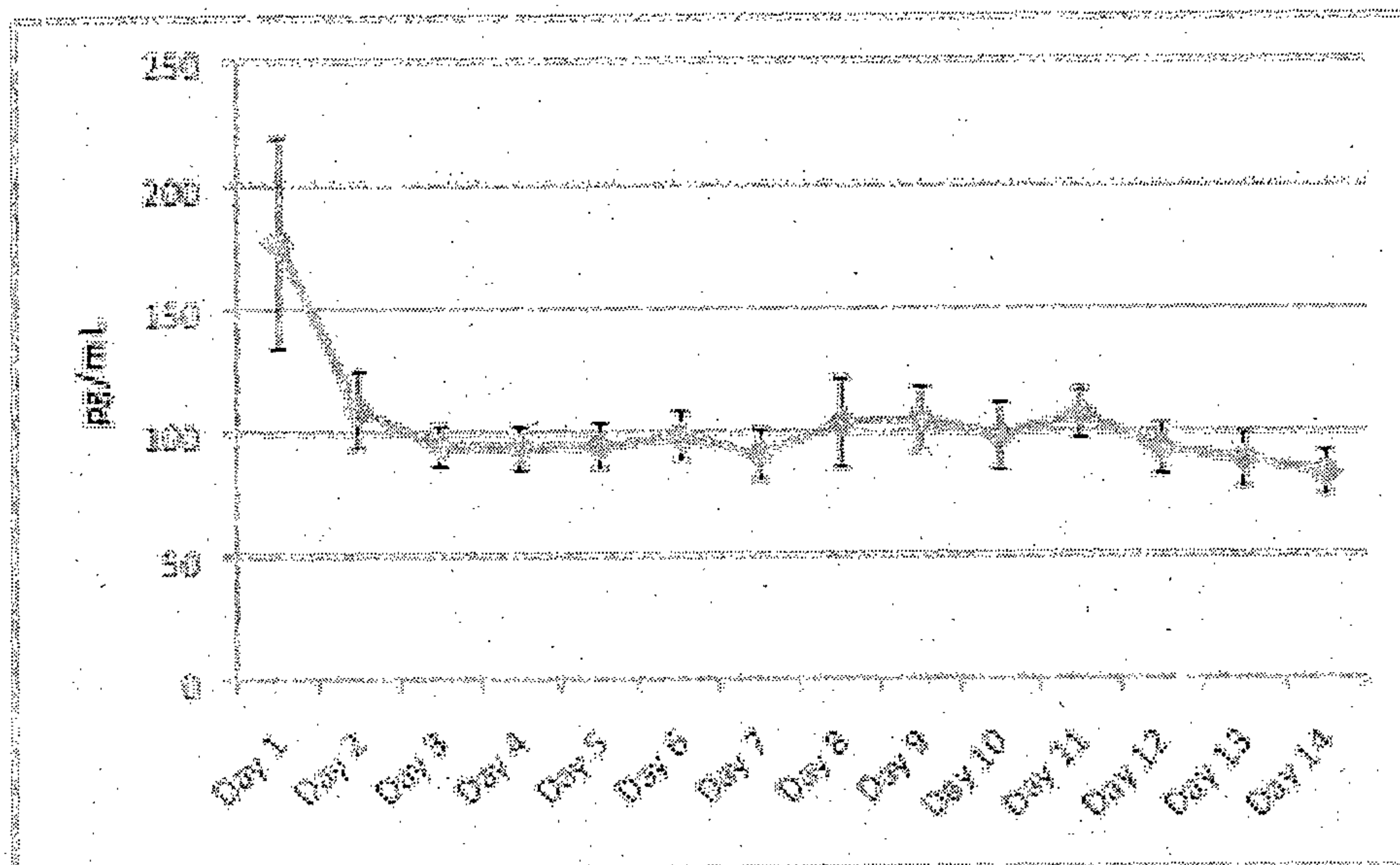


FIG. 4a (IL-1ra)

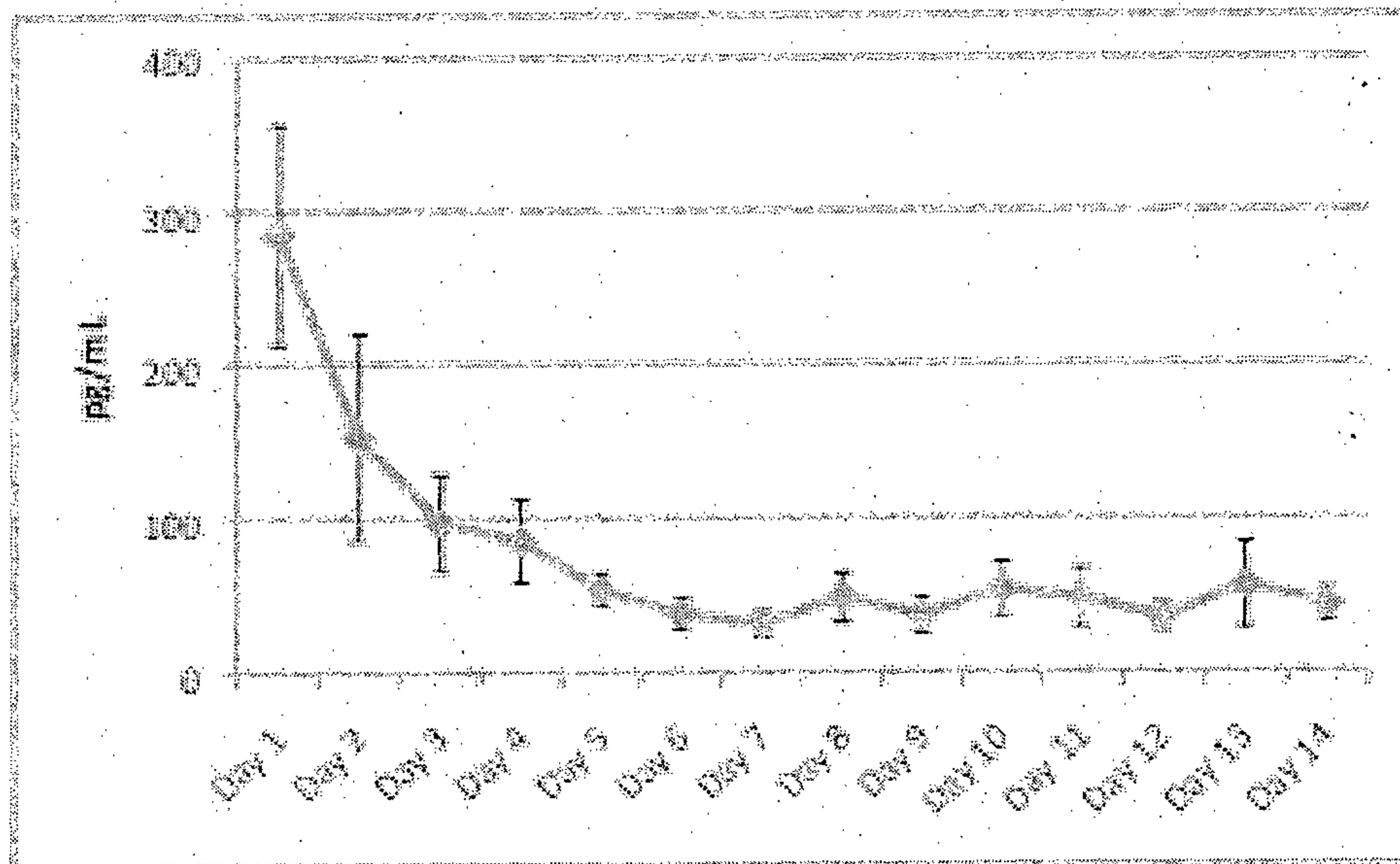


FIG. 4b (IL-6)

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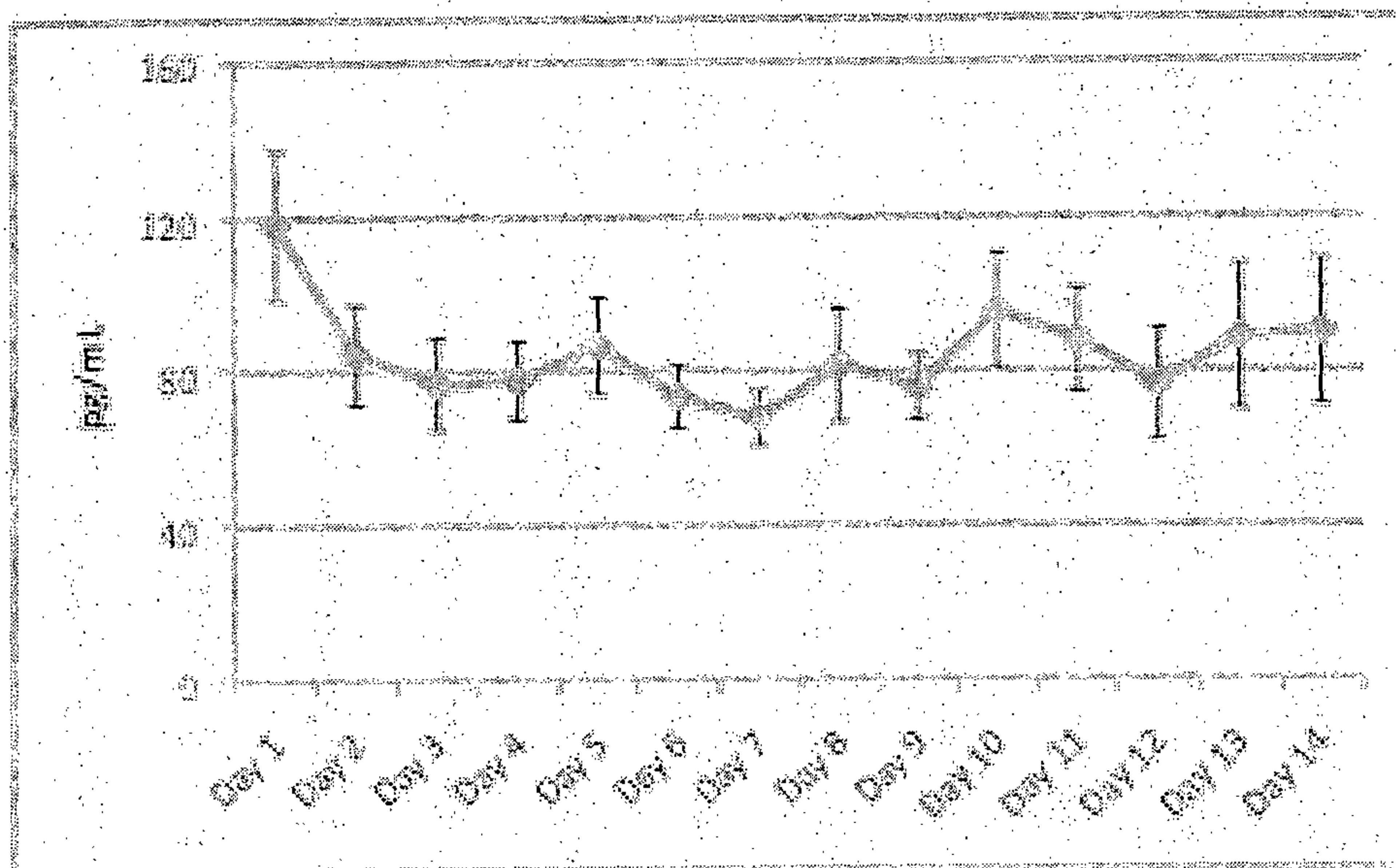


FIG. 4c (IL-8)

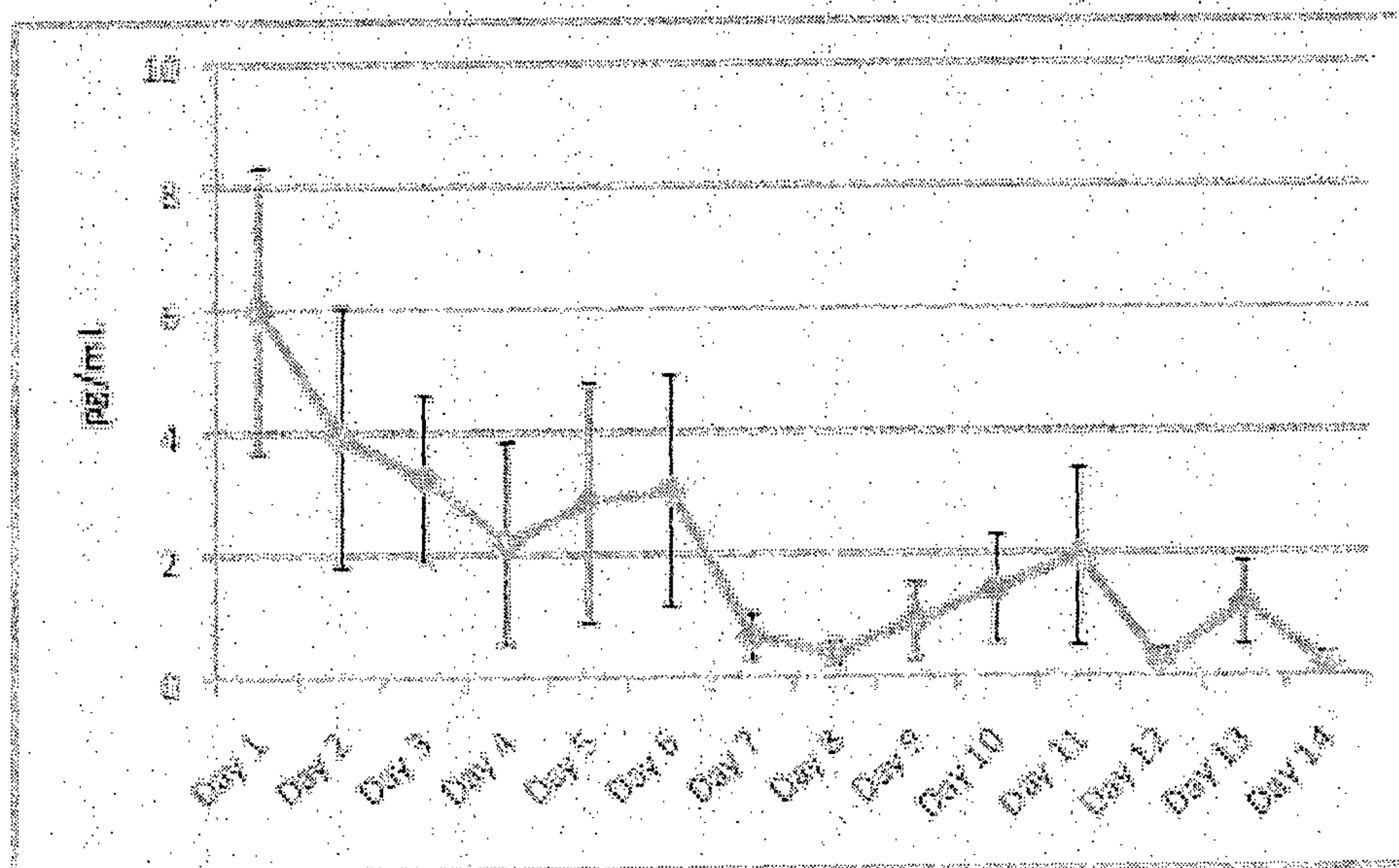


FIG. 4d (IL-15)

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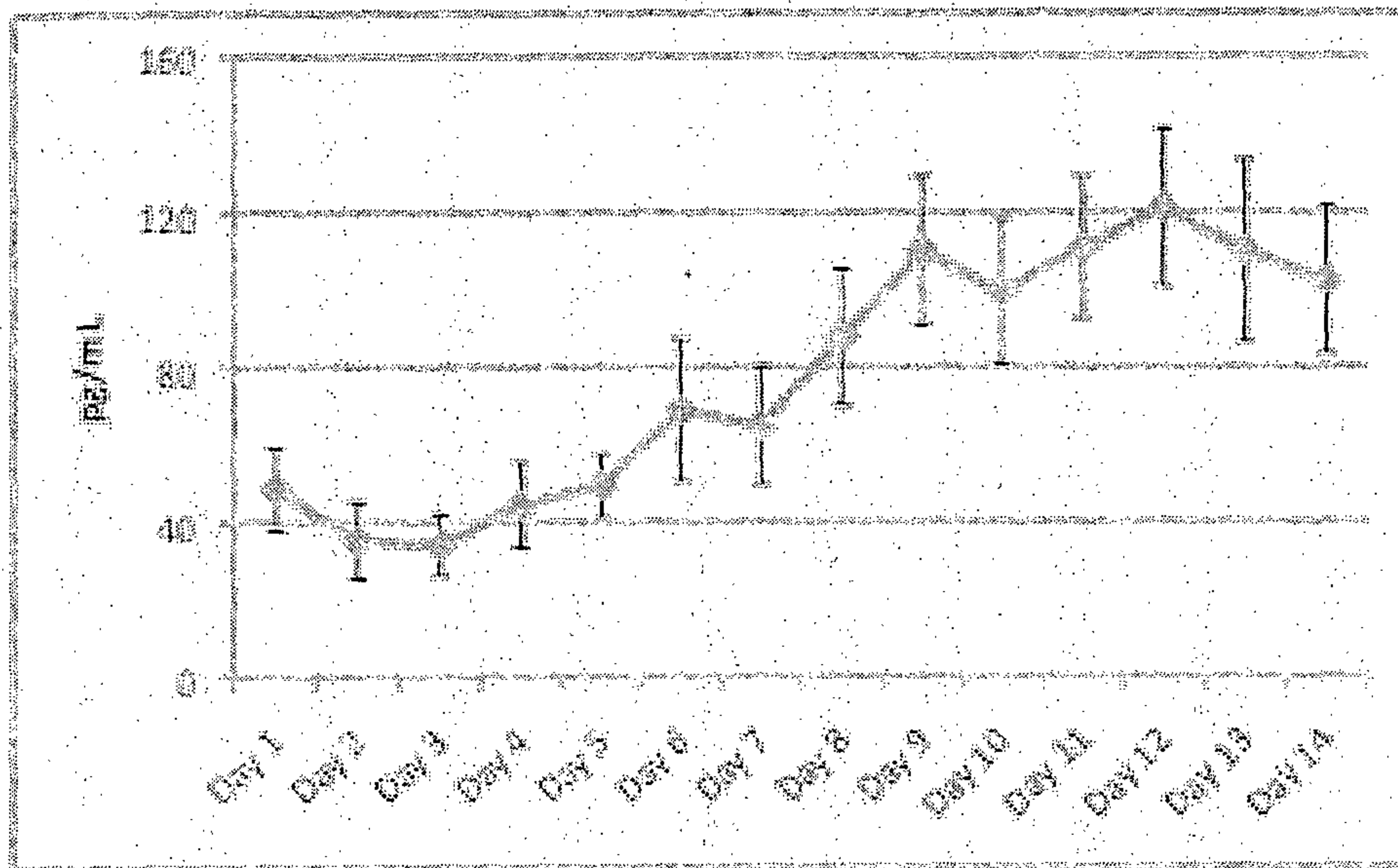


FIG. 4e (Eotaxin)

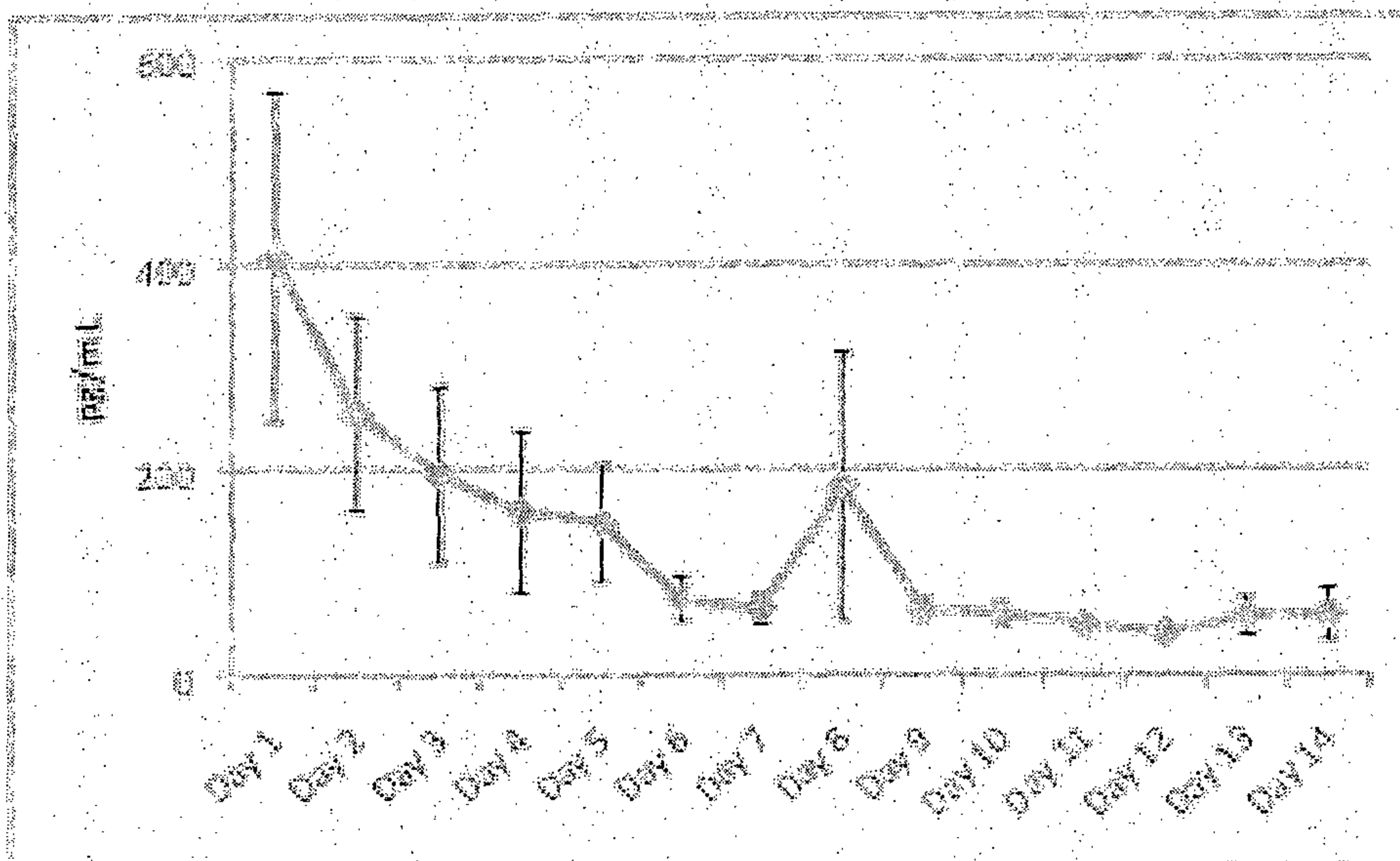


FIG. 4f (MCP-1)

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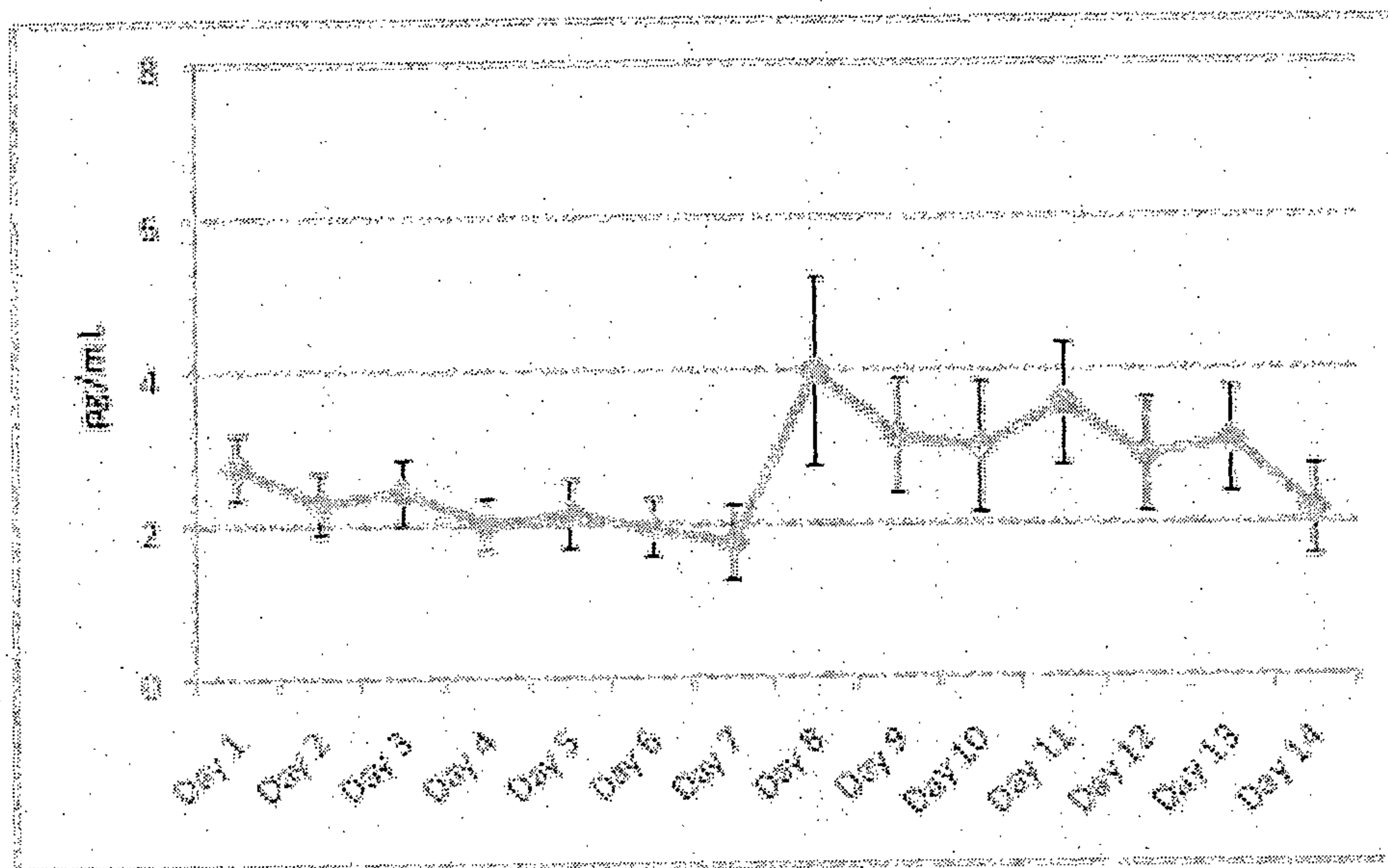


FIG. 4g (MIP-1a)

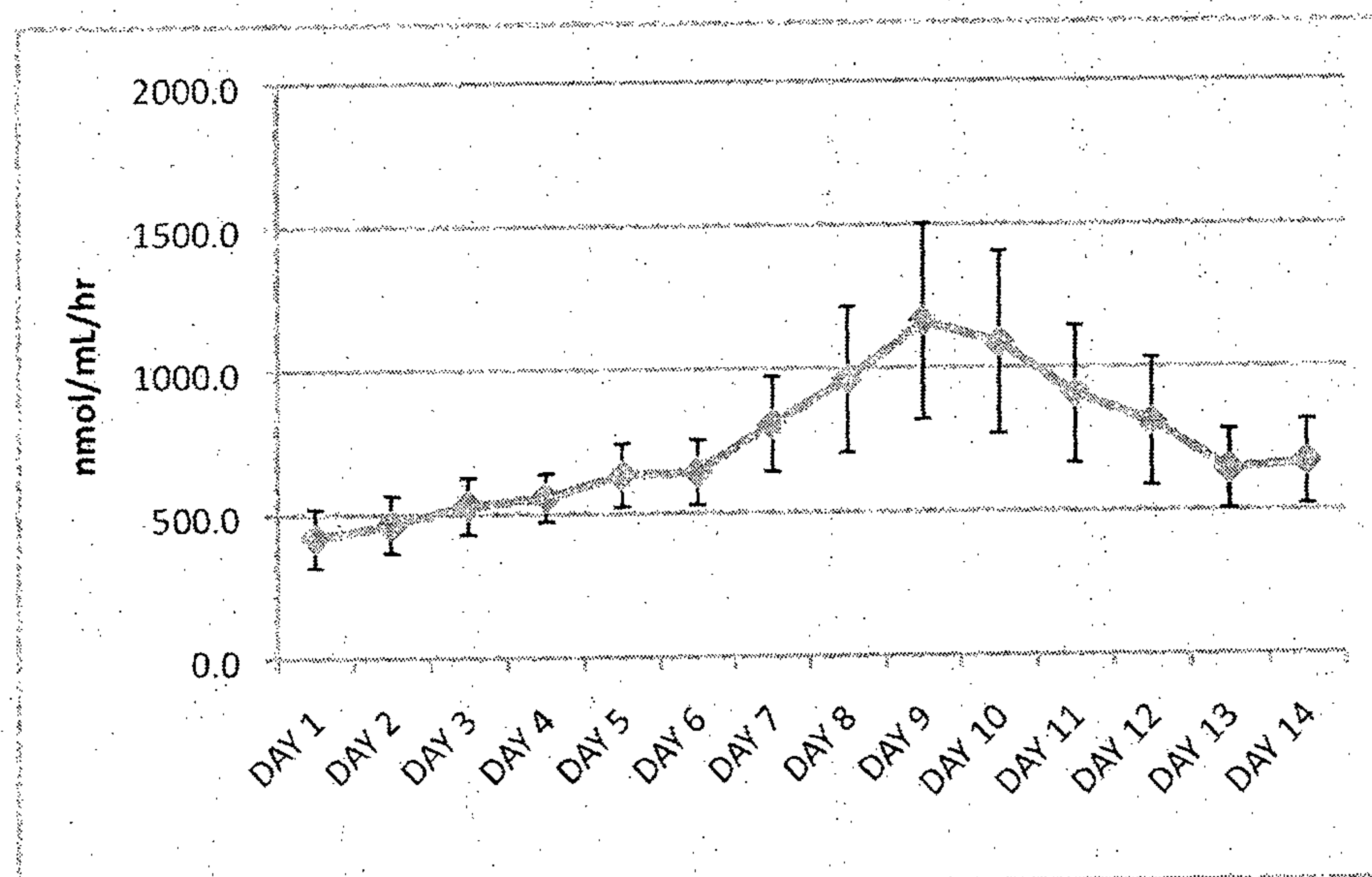


FIG. 4h (CD73)

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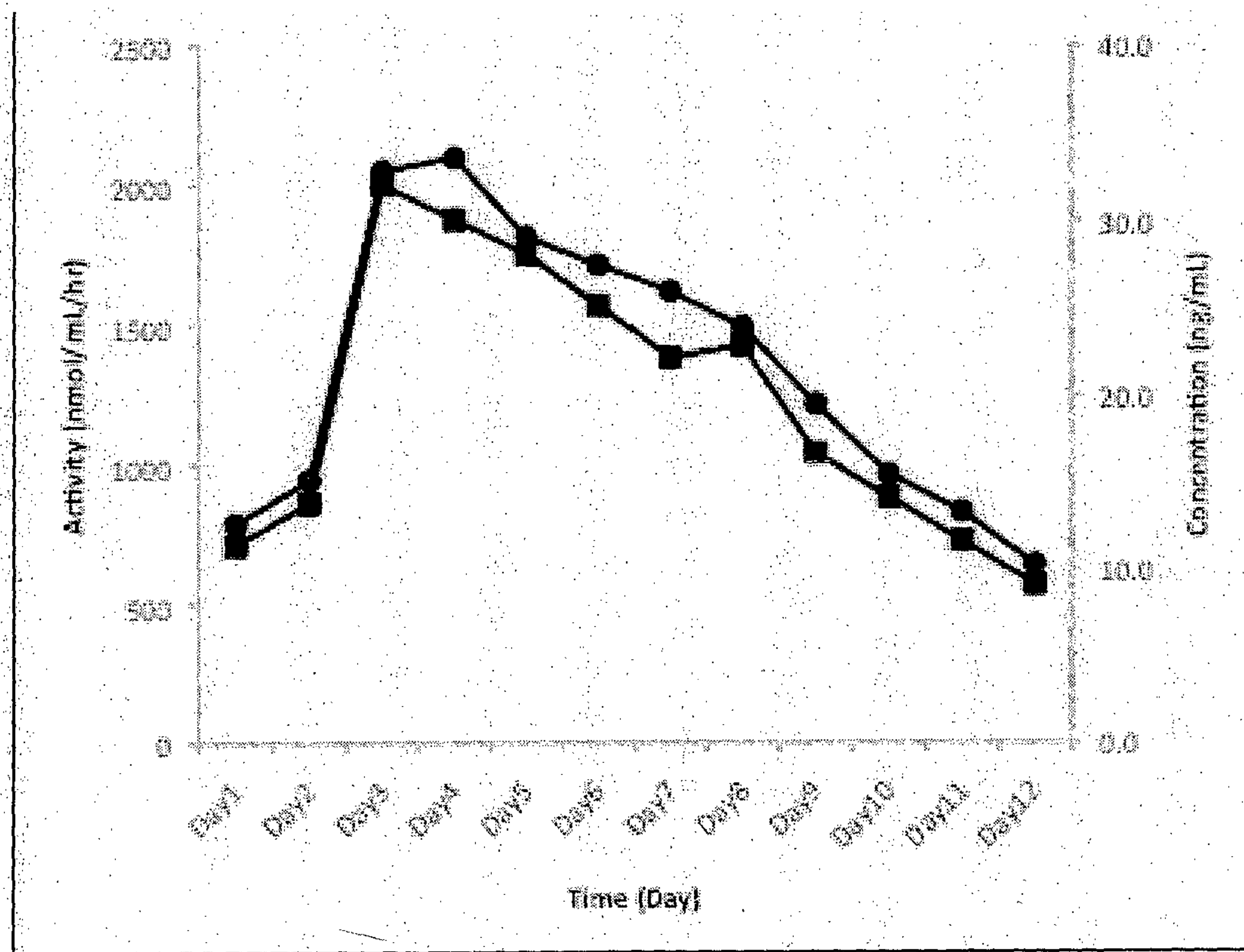


FIG. 5 (CD73)