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(19) (CA) **CANADIAN PATENT** (12)

(54) T Helper Cell Growth Factor

(72) VAN SNICK, JACQUES., BELGIUM
UYTTENHOVE, CATHERINE., BELGIUM
SIMPSON, RICHARD J., AUSTRALIA

(73) LUDWIG INSTITUTE FOR CANCER RESEARCH., U.S.A.

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ABSTRACT OF THE DISCLOSURE

1 The present invention relates generally to a T cell
growth factor. More particularly, the present invention
relates to a T cell growth factor which comprises a
glycoprotein which supports interleukin 2- and interleukin 4-
5 independent growth of helper T cells especially from murine
and human sources and further which is capable of augmenting
proliferation of IL3- or IL4-responsive cells. Even more
particularly, the present invention relates to the helper T
cell growth factor P40, pharmaceutical compositions thereof,
10 antibodies thereto and recombinant DNA clones thereof. The
present invention also contemplates a method for inducing the
proliferation of helper T cells as well as IL3- and
IL4-responsive cells. The helper T cells growth factor
contemplated herein is useful in the stimulation of specific
15 cells in the immune system, either alone or in combination
with IL3 or IL4.

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T CELL GROWTH FACTOR

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5 FIELD OF THE INVENTION

The present invention relates generally to a T cell growth factor. More particularly, the present invention relates to a mammalian T cell growth factor which is a glycoprotein capable of supporting interleukin 2- and
10 interleukin 4-independent growth of helper T cells. This factor is further capable of augmenting proliferation of IL3- and IL4-responsive cells. Even more particularly, the present invention relates to the helper T cell growth factor P40, pharmaceutical compositions thereof and antibodies
15 thereto. The present invention also contemplates a method for inducing the proliferation of helper T cells as well as IL3- and IL4-responsive cells. The helper T cell growth factor contemplated herein is useful in the stimulation of specific cells in the immune system.

20

BACKGROUND OF THE INVENTION

Many cytokines are polypeptides which directly or indirectly mediate host defense mechanisms and/or which mediate tissue growth differentiation. Cytokines have been
25 recognized which mediate host defense against cancer and/or infection. Such cytokines include the interferons (IFN- α , IFN- β and IFN- γ), tumor necrosis factor (TNF- α), lymphotoxin (TNF- β), the interleukins (IL1, 2, 3, 4, 5 and
30 6), leukoregulin, natural killer cell cytotoxic factor (NKCF), transforming growth factor (TGF), colony stimulating factors (CSF) such as macrophage (M-CSF), granulocyte (G-CSF)

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and macrophage, granulocyte-CSF (G,M-CSF) and oncostatin M.

1 Each of the aforementioned cytokines have unique characteristics and a unique range of antiproliferative, cytostatic, antiviral or growth regulatory activity.

5 Several cytokines are synthesized by leukocytes, commonly in response to stimulation by microorganisms, antigens or mitogens. This has been observed in vitro. Following this stimulation in cell culture, the supernatant fluid is retrieved and cytokine activity identified, isolated and further characterized. In recent years, it has become
10 increasingly clear that IL2 is not the only factor controlling T cell growth. Indeed, several cytokines, including IL4 (Fernandez-Botran et al., Proc. Natl. Acad. Sci. USA, 83: 9689-9693, 1986; Lichtman et al., Proc. Natl. Acad. Sci. USA, 84: 4293-4297, 1987), G,M-CSF (Woods et al.,
15 J. Immunol., 138: 4293-4297, 1987; Kupper et al., J. Immunol., 138: 4288-4292, 1987) and, in a human system, the combination of IL1 and IL6 (Houssiau et al., Eur. J. Immunol., 18: 653-656, 1988), have now been shown to induce
20 IL2-independent T cell proliferations. Consequently, the regulation of T cell growth is more complex than originally thought, although IL2 is a potent and broadly active T cell growth factor.

An important subset of T cells is the helper T cell (T_H). At least two types of helper T cells have been
25 identified on the basis of functional criteria. One type of T_H cell (T_H1) helps B cells in a linked, antigen-specific manner, and is required early in the response. Another type of T_H (T_H2) helps B cells in a nonlinked manner and is required later in the response.

30 Several years ago, a collection of helper T cell lines from lymph nodes of antigen-primed mice was obtained

using the procedure described by Corradin et al., J.
1 Immunol., 119: 1048-1053, 1977. These cell lines were
initiated by culture in the presence of antigen and were
subsequently maintained, without addition of exogenous growth
factors, by regular feeding with antigen and irradiated
5 splenic antigen-presenting cells. Most of these cells
produce large amounts of IL3, IL4, IL5 and IL6, but no IL2
and, therefore, belong to the T_H2 type defined by Mosmann et
al., J. Immunol., 136: 2348-2357, 1986.

In accordance with the present invention, it is
10 surprisingly discovered that two clones derived from the
above-mentioned cell lines proliferated in response to their
own conditioned medium in the absence of antigen and feeder
cells. The subject invention relates to a novel T cell
growth factor distinct from other known cytokines. The new
15 growth factor is useful as a therapeutic compound to
stimulate proliferation of helper T cells.

SUMMARY OF THE INVENTION

The present invention is directed to a mammalian
20 T cell growth factor which supports interleukin 2-independent
and interleukin 4-independent growth of helper T cells, and
is preferably obtained from mouse or human sources.

More particularly, this T cell growth factor is a
protein having the identifiable characteristics of P40,
25 derivatives or fragments thereof and the further capability
of augmenting proliferation and IL3- and IL4-responsive
cells. Methods of isolating P40, its derivatives and
fragments are also provided.

Another aspect of the present invention relates to
30 a pharmaceutical composition comprising an effective amount
of P40, a derivative or fragment thereof and a

1 pharmaceutically acceptable carrier useful in the stimulation
of specific cells in the immune system. Optionally, these
compositions may also contain IL3 or IL4.

Still another aspect of the present invention
relates to antibodies specific to P40, an antigenic
5 derivative or an antigenic fragment thereof useful in
diagnostic assays for P40.

Yet another aspect of the present invention relates
to a recombinant DNA molecule and expression vectors encoding
the polypeptide portion of mammalian P40, a derivative or a
10 fragment thereof, thereby providing a convenient source of
recombinant P40.

Still yet another aspect of the present invention
contemplates a method of proliferating helper T cells which
comprises incubating said cells with a proliferating
15 effective amount of P40 or a derivative thereof for a time
and under conditions sufficient for said cells to
proliferate.

A still further aspect of this invention relates to
a method of proliferating IL3- or IL4-responsive cells by
20 administering a combination of P40 and IL3 or IL4 to a
mammal, especially a human, for a time and under conditions
sufficient to stimulate said cells to proliferate.

BRIEF DESCRIPTION OF THE FIGURES

25 Fig. 1 is a graphical representation depicting
long-term antigen-independent T cell growth induced by helper
T cell supernatant (SN). TUC2.15 cells are grown without
feeder cells and antigen in normal medium (□), in medium
supplemented with IL2 (20 U/ml, ■) or with TUC2.15 SN
30 (5% v/v, ▲).

Fig. 2 is a graphical representation depicting
 1 purification of P40. TUC7.51 supernatant is fractioned
 sequentially on an Ultrogel AcA54 gel filtration column (A),
 a TSK-phenyl hydrophobic interaction column (B), a Mono-Q
 anion exchange column (C) and Cl-reversed phase column (D).
 5 The shaded area represents P40 activity. Molecular mass
 standards shown in panel A are bovine serum albumin
 (BSA, 67 kDa), natural IL5 (45 kDa) and recombinant mouse IL6
 (22 kDa).

Fig. 3 is a graphical representation depicting
 10 growth factor activity of purified P40. TS1 cells (3×10^3
 cells/well) are cultivated in the presence of increasing
 doses of purified P40. After 3 days, cells numbers are
 evaluated by measuring hexosaminidase levels.

Fig 4A & 4B illustrates the purity of P40 and its extent
 15 of glycosylation. Panel A is a photograph showing
 silver-stained NaDodSO₄/PAGE of purified P40. The sample is
 run under reducing conditions. Panel B is an autoradiograph
 of ¹²⁵I-P40 treated with various glycosylases. Mr of
 standards is given in kDa.

20 Fig. 5 is a graphic illustration of the sequencing
 strategy of the murine P40 gene.

Fig. 6 is a graphic illustration of the expression
 of recombinant P40 in fibroblasts.

25 Fig. 7 shows the amino acid sequence of murine P40
 obtained by chemical sequencing and the various peptides used
 in obtaining this sequence.

Fig. 8 is a graphic illustration of the separation
 of endoproteinase Asp-N peptides of Cm-P40 by RP-HPLC.

30 Fig. 9 is a graphic illustration of a
 multi-wavelength plot of the HPLC profile of Fig. 8 using a

hotodiode array detector at wavelengths of (A) 290 nm,
1 (B) 280 nm, (C) 254 nm and (D) 215 nm.

Fig. 10 is a graphic illustration of a derivative
spectral analysis of endoproteinase Asp-N peptide D1 with the
zero order spectrum indicated by (---) and the second order
5 spectrum indicated by (—).

Fig. 11 is a graphic illustration of the microbore
RP-HPLC separation of peptides of Cm-P40 derived from
digestion with various proteases. Panel A shows the peptides
from S. aureus V8 protease digestion of Cm-P40 endoproteinase
10 Asp-N peptide D3. Panel B shows the peptides from a
chymotrypsin digestion of Cm-P40. Panel C shows the peptides
from a trypsin digestion of Cm-P40.

Fig. 12 is a graphic illustration of the elution
profile of the blocked amino terminal peptide D1 and related
15 synthetic peptides on RP-HPLC. Z indicates pyroglutamic
acid.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a mammalian T cell
20 growth factor which comprises a protein which supports, or is
capable of supporting, interleukin 2 (IL2)-independent and
interleukin 4 (IL4)-independent growth of helper T cells in
the absence of antigen. In accordance with the present
invention and using the methods contained herein, said T cell
25 growth factor is biologically pure. By biologically pure is
meant a composition comprising said T cell growth factor.
The composition may comprise homogeneous T cell growth factor
or may consist essentially of T cell growth factor. As used
in the specification and appended claims, supporting
30 IL2-independent and IL4-independent growth of helper T cells
refers to the ability for said cells to proliferate in the

absence of IL2 and/or IL4. This feature distinguishes the
1 subject growth factor from others presently known. In
accordance with the present invention, this ability is due to
a novel and heretofore unknown T cell growth factor.

Hereinafter, said growth factor is referred to as P40. As
5 defined herein, derivatives of P40 encompass synthetic and
naturally occurring amino acid substitutions, deletions
and/or insertions as will be apparent to the skilled artisan.
For example, non-essential amino acid deletions, i.e.,
deletion of amino acids which do not affect the activity of
10 P40 are obtainable by genetic engineering means.

Furthermore, fragments of P40 are contemplated by
the present invention. These fragments are peptides obtained
from the P40 protein and may be prepared by proteolysis of
purified P40. The peptides are purified by conventional
15 means such as HPLC chromatography and the like, and are
useful in determining the P40 amino acid sequence in
preparing antibodies to specific domains of P40 and in
identifying the P40 domains involved in stimulating T cell
growth.

20 An antigenic derivative of P40 is defined to be a
portion of P40 which is capable of reacting with an antibody
specific to P40. All such derivatives are encompassed by the
subject invention.

Accordingly, P40 is a protein, and more
25 particularly, a glycoprotein, capable of supporting long-term
IL2-independent and IL4-independent growth of helper T cell
lines in the absence of antigen, and is isolated from helper
T cell lines, especially mammalian lines like murine and
human helper T cell lines. P40 is functionally distinct from
30 all known interleukins and colony-stimulating factors. P40
is purified from the supernatant (SN) of lectin-stimulated

1 mouse helper T cell lines to a specific activity of from
about 10 U/mg to about 10^{10} U/mg, but generally to about
10⁸ U/mg and characterized as a basic (pI 10 for murine P40)
single chain protein with a Mr of from about 30 to about
40 kDa.

5 P40 can be purified from the supernatant fluid of
antigen stimulated mouse helper T cell clones (TUC2.15 and
TUC7.51). Briefly, the supernatant fluid is concentrated and
applied to a TSK-Phenyl chromatography column. Fractions
with growth factor activity on factor-dependent TS1 cells are
10 pooled, further fractionated on a *Mono-Q chromatography
column, and the resulting active fractions applied to a C1
reversed-phase HPLC column. Pure murine P40 is eluted at a
concentration of about 35% acetonitrile.

15 Two observations indicate that P40 is a
glycoprotein: (i) its heterogeneous migration pattern in
NaDodSO₄/PAGE and (ii) its binding to lentil lectin, which
points to the presence of N-linked carbohydrate side chains.
Consistent with this observation, a number of potential
N-glycosylation sites (Asn-X-Thr motif) have been identified
20 in the protein sequence determination. Moreover, additional
evidence for extensive glycosylation of the molecule is
obtained in experiments with N-glycanase treatment, which
reduced the Mr of P40 to about 15 kDa. P40 is a stable
molecule whose biological activity is not altered after
25 exposure to NaDodSO₄, acid pH or acetonitrile. By contrast,
its activity is destroyed by 2-mercaptoethanol, which
suggests that intramolecular disulfide bridges play an
important role in maintaining appropriate folding of the
molecule. P40 is also distinguished from known proteins on
30 the basis of its complete amino acid sequence. The DNA and

35 *Trade mark

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1 amino acid sequence of murine P40 and human P40 are described
herein and indicate that the two proteins are 55% homologous.

5 In addition to the aforementioned distinguishing
structural characteristics of P40, it also differs
functionally from IL2. P40 is completely inactive on
cytolytic T cell clones under conditions where their response
to IL2 is very strong; conversely, IL2 fails to support
long-term antigen independent growth of helper T cell lines,
whereas P40 is very active in this system. To date,
long-term growth of helper cells in response to P40 means
10 greater than two months and may be indefinite. In contrast
with these differences, a correlation is observed between the
sensitivity of helper T cell lines to P40 and IL4, indicating
that T cell activation by these two molecules is similarly
regulated. However, the range of activities of IL4, which
15 also stimulates the growth of a variety of IL3-dependent cell
lines and of cytolytic T cells (Mosmann et al., Proc. Natl.
Acad. Sci. USA, 83: 5654-5658, 1986; Widmer et al., Nature,
326: 795-798, 1987) is broader than that of P40, indicating
that the functional overlap between the two factors, IL4 and
20 P40, is only partial.

Another advantage of the subject T cell growth
factor, P40, is the surprising discovery that P40 is specific
for helper T cell lines. This indicates the existence of a
growth-stimulatory mechanism restricted to the helper T cell
25 subset. Such a mechanism is important for maintaining the
balance between the supply of helper T cell products like IL2
and IL4 and their increased consumption by other lymphocytes
activated in the course of the immune response.

30 While investigating the range of P40 activity, it
was surprisingly discovered that P40 augments proliferation
of IL3- or IL4-responsive cells in a synergistic manner. As

used herein, IL3- and IL4-responsive cells are immune system
 1 cells which proliferate in response to IL3 or IL4,
 respectively. These cells may include IL3-dependent cells or
 IL4-dependent cells, but are not limited thereto.
 IL3-responsive cells include helper T cells, stem cells, mast
 5 cells, eosinophils, neutrophils, monocytes, megakaryocytes,
 basophils and erythropoid cells. IL4-responsive cells
 include helper T cells, activated cytotoxic T cells,
 macrophages, mast cells and B cells (Smith, K.A.,
Biotechnology, 7: 661-667, 1989).

10 Hence there is a strong synergism with respect to
 growth for cells stimulated with P40 and IL3, or P40 and IL4.
 In a thymidine uptake assay which measures cellular
 proliferation, the combination of cytokines P40 and IL3, or
 P40 and IL4, can stimulate thymidine uptake by a factor
 15 ranging from about 4 to 40 above the stimulatory effect of
 any one of the cytokines. In general, the synergism between
 P40 and IL3, or P40 and IL4, is dose dependent and cell line
 dependent. For these proteins and a given cell line,
 suboptimal doses of P40 range from about 1-25% of optimal P40
 20 doses, suboptimal doses of IL4 range from about 5-30% of
 optimal IL4 doses, and close to optimal doses of IL3 range
 from about 70-100% of optimal IL3 doses. This synergism
 provides a further method to stimulate proliferation of IL3-
 and IL4-responsive cells, especially helper T cells, and is
 25 therapeutically useful in treating immune deficiencies,
 especially those diseases or disease states which benefit
 from proliferation of specific immune cells such as AIDS, or
 even from general proliferation of immune cells.

30 It is within the scope of the present invention to
 include biologically pure P40 in addition to homogeneous and
 heterogeneous compositions thereof. Thus in accordance with

the present invention, supernatant (SN) from a helper T cell
1 line not requiring antigen or feeders comprises P40. This SN
is able to induce cell proliferation without further
requirement for antigen or feeder cells. As further
described in Example 1, the proliferation activity is not
5 inhibited by either anti-IL4 or anti-IL2 receptor antibodies,
indicating that said activity is mediated neither directly
nor indirectly by these molecules. The active ingredient in
the aforementioned SN is shown to be, in accordance with the
present invention, P40. The SN is active on the test cells,
10 TS1, inducing half-maximal proliferation at dilutions ranging
of from about 10^{-6} to about 10^{-2} (v/v), and generally ranging
of from about 10^{-5} to about 10^{-4} (v/v). Accordingly, in
accordance with the present invention, the novel T cell
growth factor P40 is active in biologically pure form and in
15 homogeneous and heterogeneous compositions. As exemplified
herein, SN fluid is a form of heterogeneous composition of
P40. Homogeneous compositions are exemplified herein to
include pharmaceutical compositions containing homogeneous
preparations of P40, its active derivatives or fragments, and
20 the like.

The T cell growth factor P40 is contemplated herein
to be useful in stimulating the proliferation of T helper
cells in mammals. In a preferred embodiment, P40 is
particularly useful in stimulating certain subsets of T
25 helper cells in mammals. Accordingly, P40 is a new and
useful therapeutic compound capable of stimulating specific
cells within the immune cells. For example, this is
particularly important for human patients carrying defects in
certain subsets of T helper cells as may be the case with
30 various AIDS patients or immune compromised patients. It
should also be noted that of the many advantages of the

present invention, the proliferation of helper T cells by P40
1 will have the additional effect of allowing increased amounts
of other cytokines to be produced. Accordingly, the present
invention also contemplates a method of treatment of immune
deficiency comprising the administration of a proliferating
5 effective amount of P40, an active derivative, or an active
fragment thereof, for a time and under conditions sufficient
to effect proliferation of helper T cells. In accordance
with the present invention, the time required for the
proliferation of helper T cells ranges from about two days to
10 about seven days.

Accordingly, the subject invention contemplates a
method for inducing and maintaining the proliferation of
helper T cells, and preferably, certain subsets thereof, in a
mammal which comprises administering to said mammal a
15 proliferating-effective amount of a pharmaceutical
composition containing P40, an active derivative or fragment
thereof, for a time and under conditions sufficient for said
cells to proliferate. Additionally, a method for inducing
and maintaining the proliferation of helper T cells, and
20 preferably certain subsets thereof, in a mammal, is
contemplated by this invention in which a nucleic acid
molecule encoding P40 is introduced into a T cell in such a
manner that said nucleic acid molecule is expressed
intracellularly, but extrachromosomally of said cell or
25 following integration into the genome of said cell. In this
case, the nucleic acid molecule is carried to said T cell and
transferred into said cell by a second nucleic acid molecule
(e.g., various viruses). The first nucleic acid molecule is
manipulated such that it contains the appropriate signals for
30 expression. That is, in accordance with the present
invention, a method for proliferating T helper cells in a

1 mammal is contemplated comprising administering a first
nucleic acid molecule encoding P40, said nucleic acid
molecule being contained in a pharmaceutically acceptable
second nucleic acid carrier molecule such that said first
nucleic acid molecule enters a T cell and is either
5 maintained extrachromosomally or integrates into the genome
of said target all in such a manner that said first nucleic
acid molecule is expressed so as to produce an effective
amount of P40. By nucleic acid molecule is meant the
nucleotide sequence which encodes, directly or indirectly,
10 P40 or a derivative thereof. A nucleic acid molecule is
defined herein to mean RNA or DNA.

The active ingredients of a pharmaceutical
composition comprising P40 are contemplated to exhibit
excellent and effective therapeutic activity, for example, in
15 the treatment of immune compromised diseases in mammals.
Thus the active ingredients of the therapeutic compositions
comprising P40 exhibit helper T cell proliferative activity
when administered in therapeutic amounts which depend on the
particular disease. For example, from about 0.5 ug to about
20 2000 mg per kilogram of body weight per day may be
administered. The dosage regimen may be adjusted to provide
the optimum therapeutic response. For example, several
divided doses may be administered daily or the dose may be
proportionally reduced as indicated by the exigencies of the
25 therapeutic situation. A decided practical advantage is that
the active compound may be administered in a convenient
manner such as by the oral, intravenous (where water
soluble), intramuscular, subcutaneous, intranasal,
intradermal or suppository routes. Depending on the route of
30 administration, the active ingredients which comprise P40 may
be required to be coated in a material to protect said

1 ingredients from the action of enzymes, acids and other
natural conditions which may inactivate said ingredients.
For example, the low lipophilicity of P40 may allow it to be
destroyed in the gastrointestinal tract by enzymes capable of
cleaving peptide bonds and in the stomach by acid hydrolysis.
5 In order to administer P40 by other than parenteral
administration, P40 should be coated by, or administered
with, a material to prevent its inactivation. For example,
P40 may be administered in an adjuvant, co-administered with
enzyme inhibitors or in liposomes. Adjuvants contemplated
10 herein include resorcinols, non-ionic surfactants such as
polyoxyethylene oleyl ether and n-hexadecyl polyethylene
ether. Enzyme inhibitors include pancreatic trypsin
inhibitor, diisopropylfluorophosphate (DFP) and trasylol.
Liposomes include water-in-oil-in-water P40 emulsions as well
15 as conventional liposomes.

The active compounds may also be administered
parenterally or intraperitoneally. Dispersions can also be
prepared in glycerol, liquid polyethylene glycols, and
mixtures thereof, and in oils. Under ordinary conditions of
20 storage and use, these preparations contain a preservative to
prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable
use include sterile aqueous solutions (where water soluble)
or dispersions and sterile powders for the extemporaneous
25 preparation of sterile injectable solutions or dispersion.
In all cases the form must be sterile and must be fluid to
the extent that easy syringability exists. It must be stable
under the conditions of manufacture and storage and must be
preserved against the contaminating action of microorganisms
30 such as bacteria and fungi. The carrier can be a solvent or
dispersion medium containing, for example, water, ethanol,

1 polyol (for example, glycerol, propylene glycol, liquid
polyethylene glycol, and the like), suitable mixtures
thereof and vegetable oils. The proper fluidity can be
maintained, for example, by the use of a coating such as
lecithin, by the maintenance of the required particle size in
5 the case of dispersion and by the use of surfactants. The
preventions of the action of microorganisms can be brought
about by various antibacterial and antifungal agents, for
example, parabens, chlorobutanol, phenol, sorbic acid,
thimerosal, and the like. In many cases, it will be
10 preferable to include isotonic agents, for example, sugars or
sodium chloride. Prolonged absorption of the injectable
compositions can be brought about by the use in the
compositions of agents delaying absorption, for example,
aluminum monostearate and gelatin.

15 Sterile injectable solutions are prepared by
incorporating the active compounds in the required amount in
the appropriate solvent with various of the other ingredients
enumerated above, as required, followed by filtered
sterilization. Generally, dispersions are prepared by
20 incorporating the various sterilized active ingredient into a
sterile vehicle which contains the basic dispersion medium
and the required other ingredients from those enumerated
above. In the case of sterile powders for the preparation of
sterile injectable solutions, the preferred methods of
25 preparation are vacuum-drying and the freeze-drying technique
which yield a powder of the active ingredient plus any
additional desired ingredient from previously sterile-
filtered solution thereof.

30 When P40 is suitably protected as described above,
the active compound may be orally administered, for example,
with an inert diluent or with an assimilable edible carrier,

or it may be enclosed in hard or soft shell gelatin capsule,
1 or it may be compressed into tablets, or it may be
incorporated directly with the food of the diet. For oral
therapeutic administration, the active compound may be
incorporated with excipients and used in the form of
5 ingestible tablets, buccal tablets, troches, capsules,
elixirs, suspensions, syrups, wafers, and the like. Such
compositions and preparations should contain at least 1% of
active compound. The percentage of the compositions and
preparations may, of course, be varied and may conveniently
10 be between about 5 to about 80% of the weight of the unit.
The amount of active compound in such therapeutically useful
compositions is such that a suitable dosage will be obtained.
Preferred compositions or preparations according to the
present invention are prepared so that an oral dosage unit
15 form contains between about 10 ug and 1000 ug of active
compound.

The tablets, troches, pills, capsules, and the
like, may also contain the following: a binder such as gum
gragacanth, acacia, corn starch or gelatin; excipients such
20 as dicalcium phosphate; a disintegrating agent such as corn
starch, potato starch, alginic acid, and the like; a
lubricant such as magnesium stearate; and a sweetening agent
such as sucrose, lactose or saccharin may be added or a
flavoring agent such as peppermint, oil of witnergreen or
25 cherry flavoring. When the dosage unit form is a capsule, it
may contain, in addition to materials of the above type, a
liquid carrier. Various other materials may be present as
coatings or to otherwise modify the physical form of the
dosage unit. For instance, tablets, pills or capsules may be
30 coated with shellac, sugar or both. A syrup or elixir may
contain the active compound, sucrose as a sweetening agent,

1 methyl and propylparabens as preservatives, a dye and
flavoring such as cherry or orange flavor. Of course, any
material used in preparing any dosage unit form should be
pharmaceutically pure and substantially non-toxic in the
amounts employed. In addition, the active compound may be
5 incorporated into sustained-release preparations and
formulations.

It is especially advantageous to formulate
parenteral compositions in dosage unit form for ease of
administration and uniformity of dosage. Dosage unit form as
10 used herein refers to physically discrete units suited as
unitary dosages for the mammalian subjects to be treated;
each unit containing a predetermined quantity of active
material calculated to produce the desired therapeutic effect
in association with the required pharmaceutical carrier. The
15 specification for the novel dosage unit forms of the
invention are dictated by and directly dependent on (a) the
unique characteristics of the active material and the
particular therapeutic effect to be achieved, and (b) the
limitations inherent in the art of compounding such an active
20 material for the treatment of disease in living subjects
having a diseased condition in which bodily health is
impaired as herein disclosed in detail.

The principal active ingredient is compounded for
convenient and effective administration in effective amounts
25 with a suitable pharmaceutically acceptable carrier in dosage
unit form as hereinbefore disclosed. A unit dosage form can,
for example, contain the principal active compound in amounts
ranging from 0.5 ug to about 2000 mg. Expressed in
proportions, the active compound is generally present in from
30 about 10 ug to about 2000 mg/ml of carrier. In the case of
compositions containing supplementary active ingredients, the

1 dosages are determined by reference to the usual dose and
manner of administration of the said ingredients.

As used herein "pharmaceutically acceptable
carrier" includes any and all solvents, dispersion media,
coatings, antibacterial and antifungal agents, isotonic and
5 absorption delaying agents, and the like. The use of such
media and agents for pharmaceutical active substances is well
known in the art. Except insofar as any conventional media
or agent is incompatible with the active ingredient, use
thereof in the therapeutic compositions is contemplated.
10 Supplementary active ingredients can also be incorporated
into the compositions.

A further aspect of this invention contemplates the
use of P40 with IL3 or IL4 in a method to stimulate
proliferation of IL3- or IL4-responsive cells and in a method
15 of treatment of immune deficiency. Such methods are
practiced in accordance with the therapeutic methods
involving only P40 and as described herein. Likewise,
pharmaceutical compositions containing P40 and IL3, or P40
and IL4 are provided in accordance with those which contain
20 P40 alone. Further in this regard, IL3 and IL4 are
commercially available and are used in therapeutically
effective amounts. Pharmaceutically effective amounts of P40
when used in conjunction with IL3 or IL4 are the same as when
P40 is used alone. Likewise, pharmaceutically effective
25 amounts of IL3 or IL4 amounts can be similar to those
provided for P40 alone. Preferred compositions of P40 and
IL3 according to the present invention are prepared so that a
unit dosage form contains each protein in an amount ranging
from about 0.5 ug to about 2000 mg. Preferred compositions
30 of P40 and IL4 are likewise prepared so that a unit dosage
form contains each protein in an amount ranging from about

0.5 ug to about 2000 mg. In these compositions, the relative
1 amount of P40 to IL3 or IL4 can be varied or the same.

The present invention also relates to antibodies to
P40, its derivatives or fragments. Such antibodies are
contemplated to be useful in developing detection assays
5 (immunoassays) for P40, especially during the monitoring of a
therapeutic regimen and in the purification of P40. The
antibodies may be monoclonal or polyclonal. Additionally, it
is within the scope of this invention to include any second
antibodies (monoclonal or polyclonal) directed to the first
10 antibodies discussed above. The present invention further
contemplates use of these second antibodies in detection
assays and, for example, in monitoring the effect of an
administered pharmaceutical preparation. Furthermore, it is
within the scope of the present invention to include
15 antibodies to the glycosylated regions of P40, and to any
molecules complexed with said P40. Accordingly, in
accordance with this invention, an antibody to P40
encompasses antibodies to P40, or antigenic parts thereof,
and to any associated molecules (e.g., glycosylated regions,
20 lipid regions, carrier molecules, and the like).

The P40, or parts thereof, considered herein are
purified, as exemplified in Example 3, then utilized in
antibody production. Both polyclonal and monoclonal
antibodies are obtainable by immunization with P40, its
25 derivatives, polypeptides or fragments, and either type is
utilizable for immunoassays. The methods of obtaining both
types of sera are well known in the art. Polyclonal sera are
less preferred, but are relatively easily prepared by
injection of a suitable laboratory animal with an effective
30 amount of the purified P40, or parts thereof, collecting
serum from the animal and isolating specific sera by any of

1 the known immunoadsorbent techniques. Although antibodies
produced by this method are utilizable in virtually any type
of immunoassay, they are generally less favored because of
the potential heterogeneity of the product.

5 The use of monoclonal antibodies in the present
immunoassay is particularly preferred because of the ability
to produce them in large quantities and the homogeneity of
the product. The preparation of hybridoma cell lines for
monoclonal antibody production derived by fusing an immortal
10 cell line and lymphocytes sensitized against the immunogenic
preparation can be done by techniques which are well known to
those who are skilled in the art. (See, for example,
Douillard, J.Y. and Hoffman, T., "Basic Facts About
Hybridomas," in Compendium of Immunology, Vol. II, L.
Schwartz (Ed.) (1981); Kohler, G. and Milstein, C., Nature,
15 256: 495-497 (1975); European Journal of Immunology,
6: 511-519 (1976), Koprowski et al., U.S. Patent 4,172,124,
Koprowski et al., U.S. Patent 4,196,265 and Wands, U.S.
Patent 4,271,145.

20 Unlike preparation of polyclonal sera, the choice
of animal for monoclonal antibody production is dependent on
the availability of appropriate immortal lines capable of
fusing with lymphocytes thereof. Mouse and rat have been the
animals of choice in hybridoma technology and are preferably
25 used. Humans can also be utilized as sources for sensitized
lymphocytes if appropriate immortalized human (or nonhuman)
cell lines are available. For the purpose of the present
invention, the animal of choice may be injected with from
about 1 mg to about 20 mg of the purified P40 or parts
30 thereof. Usually the injecting material is emulsified in
Freund's complete adjuvant. Boosting injections may also be

1 required. The detection of antibody production can be
carried out by testing the antisera with appropriately
labeled antigen. Lymphocytes can be obtained by removing the
spleen or lymph nodes of sensitized animals in a sterile
fashion and carrying out fusion. Alternatively, lymphocytes
5 can be stimulated or immunized in vitro, as described, for
example, in C. Reading, J. Immunol. Meth., 53: 261-291, 1982.

A number of cell lines suitable for fusion have
been developed, and the choice of any particular line for
hybridization protocols is directed by any one of a number of
10 criteria such as speed, uniformity of growth characteristics,
deficiency of its metabolism for a component of the growth
medium, and potential for good fusion frequency.

Intraspecies hybrids, particularly between like
strains, work better than interspecies fusions. Several cell
15 lines are available, including mutants selected for the loss
of ability to secrete myeloma immunoglobulin. Included among
these are the following mouse myeloma lines: MPC₁₁-X45-6TG,
P3-NS1-1-Ag4-1. P3-X63-Ag8, or mutants thereof such as
X63-Ag8.653, SP2-0-Ag14 (all BALB/C derived), Y3-'Ag1.2.3
20 (rat) and U266 (human).

Cell fusion can be induced either by virus, such as
Epstein-Barr or Sendai virus, or polyethylene glycol.
Polyethylene glycol (PEG) is the most efficacious agent for
the fusion of mammalian somatic cells. PEG itself may be
25 toxic for cells, and various concentrations should be tested
for effects on viability before attempting fusion. The
molecular weight range of PEG may be varied from 1000 to
6000. It gives best results when diluted to about 20% to
about 70% (w/w) in saline or serum-free medium. Exposure to
30 PEG at 37°C for about 30 seconds is preferred in the present
case, utilizing murine cells. Extremes of temperature (i.e.,

about 45°C) are avoided, and preincubation of each component
1 of the fusion system at 37°C prior to fusion gives optimum
results. The ratio between lymphocytes and malignant cells
is optimized to avoid cell fusion among spleen cells and a
range of from about 1:1 to about 1:10 gives good results.

5 The successfully fused cells can be separated from
the myeloma line by any technique known by the art. The most
common and preferred method is to choose a malignant line
which is Hypoxanthine Guanine Phosphoribosyl Transferase
(HGPRT) deficient, which will not grow in an aminopterin-
10 containing medium used to allow only growth of hybrids and
which is generally composed of hypoxanthine 1.10^{-4} M,
aminopterin 1×10^{-5} M and thymidien 3×10^{-5} M, commonly
known as the HAT medium. The fusion mixture can be grown in
the HAT-containing culture medium immediately after the
15 fusion 24 hours later. The feeding schedules usually entail
maintenance in HAT medium for two weeks and then feeding with
either regular culture medium or hypoxanthine, thymidine-
containing medium.

20 The growing colonies are then tested for the
presence of antibodies that recognize the antigenic
preparation. Detection of hybridoma antibodies can be
performed using an assay where the antigen is bound to a
solid support and allowed to react to hybridoma supernatants
containing putative antibodies. The presence of antibodies
25 may be detected by "sandwich" techniques using a variety of
indicators. Most of the common methods are sufficiently
sensitive for use in the range of antibody concentrations
secreted during hybrid growth.

30 Cloning of hybrids can be carried out after 21-23
days of cell growth in selected medium. Cloning can be
performed by cell limiting dilution in fluid phase or by

1 directly selecting single cells growing in semi-solid
agarose. For limiting dilution, cell suspensions are diluted
serially to yield a statistical probability of having only
one cell per well. For the agarose technique, hybrids are
seeded in a semi-solid upper layer, over a lower layer
5 containing feeder cells. The colonies from the upper layer
may be picked up and eventually transferred to wells.

Antibody-secreting hybrids can be grown in various
tissue culture flasks, yielding supernatants with variable
concentrations of antibodies. In order to obtain higher
10 concentrations, hybrids may be transferred into animals to
obtain inflammatory ascites. Antibody-containing ascites can
be harvested 8-12 days after intraperitoneal injection. The
ascites contain a higher concentration of antibodies but
include both monoclonals and immunoglobulines from the
15 inflammatory ascites. Antibody purification may then be
achieved by, for example, affinity chromatography.

The presence of P40 contemplated herein, or
antibodies specific for same, in a patient's serum, tissue or
tissue extract, can be detected utilizing antibodies prepared
20 as above, either monoclonal or polyclonal, in virtually any
type of immunoassay. A wide range of immunoassay techniques
are available as can be seen by reference to U.S. Patent Nos.
4,016,043; 4,424,279 and 4,018,653. This, of course,
includes both single-site and two-site, or "sandwich", assays
25 of the non-competitive types, as well as in the traditional
competitive binding assays. Sandwich assays are among the
most useful and commonly used assays and are favored for use
in the present invention. A number of variations of the
sandwich assay technique exist, and all are intended to be
30 encompassed by the present invention. Briefly, in a typical
forward assay, an unlabeled antibody is immobilized in a

solid substrate and the sample to be tested brought into
1 contact with the bound molecule. After a suitable period of
incubation, for a period of time sufficient to allow
formation of an antibody-antigen secondary complex, a second
antibody, labeled with a reporter molecule capable of
5 producing a detectable signal is then added and incubated,
allowing time sufficient for the formation of a tertiary
complex of antibody-antigen-labeled antibody (e.g.,
antibody-P40-antibody). Any unreacted material is washed
away, and the presence of the antigen is determined by
10 observation of a signal produced by the reporter molecule.
The results may either be qualitative, by simple observation
of the visible signal, or may be quantitated by comparing
with a control sample containing known amounts of hapten.
Variations on the forward assay include a simultaneous assay,
15 in which both sample and labeled antibody are added
simultaneously to the bound antibody, or a reverse assay in
which the labeled antibody and sample to be tested are first
combined incubated and then added to the unlabeled surface
bound antibody. These techniques are well known to those
20 skilled in the art, and then possibly of minor variations
will be readily apparent.

In the typical forward sandwich assay, a first
antibody having specificity for P40, or antigenic parts
thereof, contemplated in this invention, is either covalently
25 or passively bound to a solid surface. The solid surface is
typically glass or a polymer, the most commonly used polymers
being cellulose, polyacrylamide, nylon, polystyrene,
polyvinyl chloride or polypropylene. The solid supports may
be in the form of tubes, beads, discs or microplates, or any
30 other surface suitable for conducting an immunoassay. The
binding processes are well-known in the art and generally

1 consist of cross-linking, covalently binding or physically
absorbing the molecule to the insoluble carrier. Following
binding, the polymer-antibody complex is washed in
preparation for the test sample. An aliquot of the sample to
be tested is then added to the solid phase complex and
5 incubated at 25°C for a period of time sufficient to allow
binding of any subunit present in the antibody. The
incubation period will vary but will generally be in the
range of about 2-40 minutes. Following the incubation
period, the antibody subunit solid phase is washed and dried
10 and incubated with a second antibody specific for a portion
of the hapten. The second antibody is linked to a reporter
molecule which is used to indicate the binding of the second
antibody to the hapten. By "reporter molecule," as used in
the present specification, is meant a molecule which, by its
15 chemical nature, provides an analytically identifiable signal
which allows the detection of antigen-bound antibody.
Detection may be either qualitative or quantitative. The
most commonly used reporter molecules in this type of assay
are either enzymes, fluorophores or radionuclide containing
20 molecules (i.e., radioisotopes). In the case of an enzyme
imunoassay, an enzyme is conjugated to the second antibody,
generally by means of glutaraldehyde or periodate. As will
be readily recognized, however, a wide variety of different
conjugation techniques exist, which are readily available to
25 the skilled artisan. Commonly used enzymes include

30

35

horseradish peroxidase, glucose oxidase, β -galactosidase and
1 alkaline phosphatase, among other. The substrates to be used
with the specific enzymes are generally chosen for the
production, upon hydrolysis by the corresponding enzyme, of a
detectable color change. For example, p-nitrophenyl
5 phosphate is suitable for the use with alkaline phosphatase
conjugates; for peroxidase conjugates, 1,2-phenylenediamine,
5-aminosalicylic acid, or toluidine, are commonly used. It
is also possible to employ fluorogenic substrates, which
yield a fluorescent product rather than the chromogenic
10 substrates noted above. In all cases, the enzyme-labeled
antibody is added to the first antibody hapten complex,
allowed to bind, and then to the first antibody hapten
complex, allowed to bind, and then the excess reagent is
washed away. A solution containing the appropriate substrate
15 is then added to the ternary complex of antibody-antigen-
antibody. The substrate will react with the enzyme linked to
the second antibody, giving a qualitative visual signal,
which may be further quantitated, usually
spectrophotometrically, to give an indication of the amount
20 of hapten which was present in the sample.

Alternately, fluorescent compounds, such as
fluorescein and rhodamine, may be chemically coupled to
antibodies without altering their binding capacity. When
activated by illumination with light of a particular
25 wavelength, the fluorochrome-labeled antibody adsorbs the
light energy, inducing a state of excitability in the
molecule, followed by emission of the light at a
characteristic color visually detectable with a light
microscope. As in the EIA, the fluorescent labeled antibody
30

is allowed to bind to the first antibody-hapten complex.

- 1 After washing off the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA
- 5 techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled
- 10 technician how to vary the procedure to suit the required purpose. It will also be apparent that the foregoing can be used to detect directly or indirectly (i.e., via antibodies) the P40 of this invention.

Accordingly, the present invention is also directed

15 to a kit for the rapid and convenient assay of P40 in mammalian body fluids (e.g. serum, tissue extracts, tissue fluids), in vitro cell culture supernatants, and cell lysates. The kit is compartmentalized to receive a first container adapted to contain an antibody to P40, or to an

20 antigenic component thereof, and a second container adapted to contain a second antibody to P40, or to an antigenic component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal as hereinbefore described. If the reporter molecule is an

25 enzyme, then a third container adapted to contain a substrate for said enzyme is provided. In an exemplified use of the subject kit, a sample to be tested for P40 is contacted with the contents of the first container for a time and under conditions for P40, if present, to bind to the antibodies

30 contained in said first container. After removal of unbound

material (e.g. by washing with sterile phosphate buffered
1 saline) the secondary complex is contacted with the contents
of the second container. If the antibodies of the first
container have bound to P40, then the antibodies of the
second container bind to the secondary complex to form a
5 tertiary complex and, since said second antibodies are labeled
with a reporter molecule, when subjected to a detecting
means, the tertiary complex is detected.

Another aspect of this invention relates to a
recombinant nucleic acid or an isolated nucleic acid
10 molecule, said molecule defined herein to be DNA or RNA,
encoding P40 or parts thereof. In one embodiment the
recombinant nucleic acid molecule is complementary DNA
(cDNA). It is considered within the scope of the present
invention to include the cDNA molecule encoding mammalian
15 P40, preferably murine and human P40, or to regions or parts
thereof including any base deletion, insertion or
substitution or any other alteration with respect to
nucleotide sequence or chemical composition (e.g. methylation
and glycosylation). P40 encoded by cDNA is referred to
20 herein as recombinant P40. Moreover, another embodiment of
this invention is directed to the genomic P40 gene, which may
include recombinant clones like cosmids encoding the entire
gene or subclones encoding exons, introns or any region of
the mammalian P40 gene. Recombinant DNA encoding such
25 subregions of the gene are useful as hybridization probes to
detect the presence of P40 genes.

Methods considered useful in obtaining recombinant
P40 cDNA are contained in Maniatis et al., 1982, in Molecular
Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory,
30 New York, pp. 1-545, for example, or any of the myriad of

laboratory manuals on recombinant DNA technology which are
 1 widely available. Briefly, polyadenylated mRNA is obtained
 from stimulated helper T cells and fractionated on agarose
 gels. Optionally, aliquots of mRNA can be injected into
Xenopus laevis oocytes for translation and assayed for P40
 5 activity using the methods contained herein to enriched
 fractions of mRNA translating into P40 active molecules.
 Alternatively, mRNA not enriched is used as template for cDNA
 synthesis. Libraries of cDNA clones are constructed in the
PstI site of the vector pBR322 (using homopolymer tailing) or
 10 in a variety of other vectors (e.g. the Okayama-Berg cDNA
 cloning vectors, Messing cDNA cloning vectors and the like).
 Specific cDNA molecules in a vector in said library is then
 selected by using specific oligonucleotides designed, based
 on amino acid sequences contained within P40, to encode at
 15 least part of said sequence. Particularly useful is the
 internal, partial amino acid sequence of murine P40 obtained
 after cyanogen bromide treatment which comprises:

20 NH₂-Ala Gly Asn Thr Leu Ser Phe Leu Lys Ser Leu
 Leu Gly Thr Phe Gln Lys Thr Glu.

Oligonucleotide sequences based on the foregoing amino acid
 sequence are particularly useful in identifying cDNA clones
 encoding P40 or its derivatives. Thus, poly(A)⁺RNA can be
 25 prepared from the murine helper T cell line TUC7.51 after 24
 hours stimulation with Concanavalin A (Con A) and used as a
 template for cDNA synthesis. The cDNA can be cloned into
BamHI site of a pUC8 vector, transformed into E. coli and
 screened using a 64-fold degenerate probe corresponding to
 30 the amino acid sequence FQKTEMQ and subsequently with a

128-fold degenerate probe corresponding to the amino acid
 1 sequence ENLKDDP (see Example 9 for the exact sequence of the
 probes). The resulting positive clones are useful to isolate
 other mammalian genomic P40 genes and cDNAs. For example,
 the murine cDNA clone is used to screen a human genomic
 5 library, or other mammalian genomic library, to identify
 either the entire genomic gene or at least an exon thereof.
 If only a portion of the gene is isolated by this method, the
 remainder of the gene can be isolated by "chromosomal
 walking" with the new clone. Further, a genomic clone is
 10 particularly useful to isolate a cDNA clone and vice versa,
 especially from the same species. Thus, the murine cDNA
 clone is used to isolate the murine genomic P40 gene.

The cDNA sequence encoding murine P40 is set forth
 below with the corresponding amino acid sequence:

15

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      -18                               -10
      MetLeuValThrTyrIleLeuAlaSerValLeuLeuPheSerSer
5' CAGACTCCCGTCAACATGTTGGTGACATACCTTGCTTCTGTTTGTCTTCAGTTCT

      1                               10
ValLeuGlyGlnArgCysSerThrThrTrpGlyIleArgAspThrAsnTyrLeuIleGlu
GTGCTGGGCCAGAGATGTCAGCACCACATGGGGCATCAGAGACACCAATTACCTTATTGAA
20
      20                               30
AsnLeuLysAspAspProProSerLysCysSerCysSerGlyAsnValThrSerCysLeu
AATCTGAAGGATGATCCACCGTCAAAATGTCAGCTGTCAGCGGCAACGTGACCAGCTGCTTG

      40                               50
CysLeuSerValProThrAspAspCysThrThrProCysTyrArgGluGlyLeuLeuGln
TGTCTCTCCGTCCCAACTGATGATTGTACCACACCGTGTACAGGGAGGGACTGTTACAG
25
      60                               70
LeuThrAsnAlaThrGlnLysSerArgLeuLeuProValPheHisArgValLysArgIle
CTGACCAATGCCACACAGAAATCAAGACTCTTGCTGTTTTCATCGGGTGAAAAGGATA
      80                               90
ValGluValLeuLysAsnIleThrCysProSerPheSerCysGluLysProCysAsnGln
GTTGAAGTCCTAAAGAACATCACGTGTCGGTCCTTTTCTGTGGAAAAGCCATGCAACCAG
      100                              110
ThrMetAlaGlyAsnThrLeuSerPheLeuLysSerLeuLeuGlyThrPheGlnLysThr
ACCATGGCAGGCAACACACTGTCATTTCTGAAGAGTCTCCTGGGGACGTTCCAGAAGACA
      120                              126
GluMetGlnArgGlnLysSerArgPro
GAGATGCAAAGGCAGAAAAGCCGACCATGAAGACAGATGCTATTTATTCTATTATTGAA
30
TTTACAAAACCTCCCCTCCTTAAGTGTACAGTGAAGAAATAAATAAGCTATTCT 3'
      500
  
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35

The cDNA sequence with the corresponding amino acid
1 sequence of human P40 is set forth below:

5
10
15
20
25
30
35

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-18
MET LEU LEU ALA MET VAL LEU THR SER ALA LEU LEU LEU CYS SER VAL ALA
CCGCTGTCAAG ATG CTT CTG GCC ATG GTC CTT ACC TCT GCC CTG CTC CTG TGC TCC GTG GCA 62
1
GLY GLN GLY CYS PRO THR LEU ALA GLY ILE LEU ASP ILE ASN PHE LEU ILE ASN LYS MET
GGC CAG GGG TGT CCA ACC TTG GCG GGG ATC CTG GAC ATC AAC TTC CTC ATC AAC AAG ATG 122
20
GLN GLU ASP PRO ALA SER LYS CYS HIS CYS SER ALA ASN VAL THR SER CYS LEU CYS LEU
CAG GAA GAT CCA GCT TCC AAG TGC CAC TGC AGT GCT AAT GTG ACC AGT TGT CTC TGT TTG 182
40
GLY ILE PRO SER ASP ASN CYS THR ARG PRO CYS PHE SER GLU ARG LEU SER GLN MET THR
GGC ATT CCC TCT GAC AAC TGC ACC AGA CCA TGC TTC AGT GAG AGA CTG TCT CAG ATG ACC 242
60
ASN THR THR MET GLN THR ARG TYR PRO LEU ILE PHE SER ARG VAL LYS LYS SER VAL GLU
AAT ACC ACC ATG CAA ACA AGA TAC CCA CTG ATT TTC AGT CGG GTG AAA AAA TCA GTT GAA 302
80
VAL LEU LYS ASN ASN LYS CYS PRO TYR PHE SER CYS GLU GLN PRO CYS ASN GLN THR THR
GTA CTA AAG AAC AAC AAG TGT CCA TAT TTT TCC TGT GAA CAG CCA TGC AAC CAA ACC ACG 362
100
ALA GLY ASN ALA LEU THR PHE LEU LYS SER LEU LEU GLU ILE PHE GLN LYS GLU LYS MET
GCA GGC AAC GCG CTG ACA TTT CTG AAG AGT CTT CTG GAA ATT TTC CAG AAA GAA AAG ATG 422
126
ARG GLY MET ARG GLY LYS ILE
AGA GGG ATG AGA GGC AAG ATA TGAAGATGAAATATTATTTATCCTATTTATTAAATTAAAAA 485

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15 Once identified, cDNAs or recombinant DNAs encoding
all or part of recombinant P40 are ligated into expression
vectors. Additional genetic manipulation is routinely
carried out to maximize expression of the cDNA in the
particular host employed. Accordingly, P40 may be
20 synthesized in vitro by inserting said cDNA sequence into a
replicable expression vector, transforming the resulting
recombinant molecule into a suitable host and then culturing
or growing the transformed host under conditions requisite for
the synthesis of the molecule. The recombinant molecule
25 defined herein should comprise a nucleic acid sequence
encoding a desired polypeptide inserted downstream of a
promoter, a eukaryotic or prokaryotic replicon and a
selectable marker such as resistance to an antibiotic.

30 A promoter consists of a specific nucleic acid
sequence that is operably linked to the DNA encoding the
desired polypeptide which is capable of effecting expression

of said polypeptide. Likewise, the promoter can be replaced
1 or augmented by any other genetic elements capable of
effecting gene expression, including such elements as
enhancers, transcription terminators, poly(A) signals and the
like. The latter three elements are not always necessary and
5 their use will depend on both the vector and host system used
for gene expression. The need for any of these elements can
be easily determined by one skilled in the art. Promoters
are DNA sequence elements for controlling gene expression, in
particular, they specify transcription initiation sites.
10 Prokaryotic promoters that are useful include the lac
promoter, the trp promoter, the P_L and P_R promoters of lambda
and the T7 polymerase promoter. Eukaryotic promoters are
especially useful in the invention and include promoters of
viral origin, such as the SV40 late promoter and the Molony
15 Leukemia Virus LTR, yeast promoters and any promoters or
variations of promoters designed to control gene expression,
including genetically-engineered promoters. Control of gene
expression includes the ability to regulate a gene both
positively and negatively (i.e., turning gene expression on
20 or off) to obtain the desired level of expression.

One skilled in the art has available many choices
or replicable expression vectors, compatible hosts and
well-known methods for making and using the vectors.
Recombinant DNA methods are found in any of the myriad of
25 standard laboratory manuals on genetic engineering.

The recombinant molecule may also require a signal
sequence to facilitate transport of the synthesized
polypeptide to the extracellular environment. Alternatively,
the polypeptide may be retrieved by first lysing the host
30 cell by a variety of techniques such as sonication, pressure

dissintegration or toluene treatment. Hosts contemplated in
1 accordance with the present invention can be selected from
the group comprising prokaryotes (e.g., Escherichia coli,
Bacillus sp., Pseudomonas sp.) and eukaryotes (e.g.,
mammalian cells, yeast and fungal cultures, insect cells and
5 plant cultures). The artisan will also recognize that a
given amino acid sequence can undergo deletions,
substitutions and additions of nucleotides or triplet
nucleotides (codons). Such variations are all considered
within the scope of the present invention and may be prepared
10 by site-directed mutagenesis technique. Additionally,
depending on the host expressing recombinant P40, said P40
may or may not be glycosylated. Generally, eukaryotic cells,
for example mammalian T cells and the like, provide
glycosylated, recombinant P40. Prokaryotic cells, for
15 example bacteria such as Escherichia coli and the like, do
not glycosylate proteins. Hence, both glycosylated and non-
glycosylated recombinant P40 are encompassed by the present
invention.

Yet another aspect of the present invention
20 provides transformant microorganisms and cultured cells
containing the instant expression-vectors. Transformant
microorganisms and cultured cells are made by introducing the
replicable expression vector encoding mammalian P40, a
derivative or a fragment thereof, into the desired cell or
25 microorganisms by transformation or transfection or infection
of virus or bacteriophage particles. Processes for
transformation are well-known in the art and include, but are
not limited to CaCl_2 treatment and electroporation for
bacterial cells and CaPO_4 co-precipitation, protoplast fusion
30 and electroporation for eukaryotic cells. Direct infection

can be used when the vectors are viruses or bacteriophages.

1 The detailed methods for these techniques can be found in
standard laboratory manuals on recombinant DNA technology.
The invention further contemplates any method for
incorporating DNA into a host organism.

5 Another aspect of the present invention relates to
the helper T cell lines which produce P40. As defined
herein, P40 or compositions comprising same, stimulate the
development of permanent antigen-independent T helper cell
lines which are maintained by subcultivation every 3 to 4
10 days in medium with P40. Even more particularly, the present
invention is directed to TS1, one of the factor-dependent
cell lines derived from TUC2.15.

The following examples further illustrate the
present invention.

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EXAMPLE 1

1

Materials and MethodsMedium

5 Dulbecco's modified Eagle's medium supplemented
with 10% (v/v) fetal bovine serum (FCS), 50 μ M
B-mercaptoethanol, 0.55 mM L-arginine, 0.24 mM L-asparagine
and 1.25 mM L-glutamine are used for most cell lines except
for 7TD1 and BCL1 which are grown in Iscove's medium.

T Cell Clones and Lines

10

Helper T cell lines are established and maintained
in the absence of exogenous growth factors as described by
Van Snick et al. Proc. Natl. Acad. Sci. USA 83:9679-9683,
1986. Lines TUC2 and TUC7 are derived from C57BL/6 mice
immunized with keyhole limpet hemocyanin. Line TUC5 is
15 obtained from the same strain of mice but after immunization
with human transferrin. TUC13 is an allospecific BALB/c
anti-C57B/6 line. Individual clones are derived from these
lines by limiting dilution in the presence of 10% (v/v)
medium conditioned by rat spleen cells stimulated with
20 concanavalin A, and are denoted TUCx.y (where x stands for
the number of the line and y for the number of the clone).
These clones are subsequently expanded and maintained without
exogenous growth factors like the parental cell lines.
Cytolytic T cell clones of DBA/2 origin directed against
25 syngeneic P815 mastocytoma are maintained with 50% (v/v)
mixed lymphocyte culture medium as described by Maryanski et
al. Eur. J. Immunol. 12:401-406, 1982. For use in growth
factor assays, the T cells are separated from feeder cells by
centrifugation over a layer of *Lymphoprep (Nycomed AS, Oslo,
30 Norway) washed and incubated at 5×10^4 cells/well.
Proliferations are measured on day 3 after a 6 hr pulse with
methyl-labeled [^{3} H]-thymidine (0.5 μ Ci/well).

35

*Trade mark

A

Preparation of Helper T Cell Supernatants

1 TUC2.15 and TUC7.51 cells, obtained from cultures
stimulated 2 weeks earlier with antigen and feeder cells, are
adjusted to 2×10^6 cells/ml and incubated for 2-3 days in
medium containing 0.5% (v/v) FCS and concanavalin A (ConA, 5
5 ug/ml). Supernatants (SN) are collected by centrifugation at
10,000 g for 20 min. When used for culture, crude SN are
supplemented with 0.1 M methyl- α -D-mannoside.

TS1 Growth Factor Assay

Factor-dependent TS1 cells are cultured in 1% (v/v)
10 TUC2.15 SN. Before use in the growth factor assay, the cells
are washed free of SN and cultured at a density of 3×10^3
cells/well in 200 ul with serial dilutions of samples to be
tested. After 3 days, cell growth is measured by
colorimetric determination of hexosaminidase levels according
15 to Landegren J. Immunol. Methods 67:379-388, 1984. The
dilution giving half-maximal absorbance at 405 nm is
arbitrarily assigned one U/ml of activity.

Other Cell Lines

CTLL-2 (Gillis et al., J. Immunol. 120:2027-2032,
20 1978) is grown with 100 U/ml of human recombinant IL-2 DA-1
(Ihle et al. Adv. Viral Oncol. 4:95-137, 1984), Ea3.15
(Palacios et al. J. Exp. Med. 152:1036-1047, 1980) with 10%
(v/v) WEHI-3 SN as a source of IL3 and 7TD1 with a 1/500
dilution of TUC2.15 SN as a source of IL6 (Van Snick et al.
25 supra). Assays using these cell lines are carried out as
described for the TS1 line and proliferations are measured
either by hexosaminidase determinations or by thymidine
incorporation. In vivo passaged BCL1 cells (Slavin et al.
Nature 272:624-626, 1978) are frozen in aliquots and thawed
30 just before use. Proliferation of BCL1 is measured by
thymidine incorporation in 7 day-old cultures seeded with 10^4
cells/well.

Cytokines and Growth Factors

1 Purified natural human IL1 β (Van Damme et al.,
Nature 314:266-268, 1985), recombinant human IL2 (Devos et
al. Nucl. Acids Res. 11:4307-4323) and purified murine IL3
(Ihle et al. J. Immunol. 129:2431-2436, 1982) are as
5 described. Human recombinant granulocyte colony-stimulating
factor (G-CSF) and mouse recombinant granulocyte-macrophage
colony stimulating factor (GM-CSF) is described by DeLamarter
et al. Embo J. 4:2575-2581, 1985. Platelet-derived growth
factor (PDGF) is described by Heldin et al. Proc. Natl. Acad.
10 Sci. USA 76:3722-3726, 1979. Epidermal growth factor (EGF)
is purchased from Boehringer Mannheim (Fed. Rep. Germany).
Mouse IL4, IL5 and IL6 are purified as described by Van
Snick, supra, and Vink et al. Eur. J. Immunol. 18:607-612,
1988.

15 Antibodies

Anti-IL4 antibody 11B11 (Ohara et al. Nature
315:333-336, 1985) and anti-IL2 receptor antibody 5A2 (Moreau
et al. Eur. J. Immunol. 17:929-935, 1987) are as described.

Purification of TSl Growth Factor

20 Adsorption to silicic acid and gel filtration is
performed as described (Van Snick supra). Active fractions
from the gel filtration column are pooled, concentrated by
ultrafiltration on an *Amicon YM-10 membrane in the presence
of 10⁻⁴ (v/v) dilution of Tween 20 and transferred to 1 M
25 Na₂SO₄ buffered to pH 7.0 with 0.1 M sodium phosphate before
injection onto a *TSK-Phenyl column (LKB, Bromma, Sweden)
equilibrated in the same buffer. After a 10 min wash in the
starting buffer, elution is carried out at 0.6 ml/min with a

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linear gradient of a 1:1 mixture of a sodium phosphate buffer
 (0.1 M pH 7.0) and ethylene glycol. Active fractions are
 further fractionated on a *MonoQ column (Pharmacia Fine
 Chemicals, Uppsala, Sweden) equilibrated in 20 mM
 ethanolamine-HCl pH 9.5, 20 mM NaCl and 10^{-4} (v/v) *Tween 20.
 5 The column is developed at 0.8 ml/min with a 30 min linear
 gradient of NaCl (8 mM/min). Pooled active fractions are
 concentrated and adjusted to contain 0.05% (w/v)
 trifluoroacetic acid (TFA) before injection on a C1 25-nm
 pore-size TSK *TMS-250 HPLC column (LKB). The column is
 10 developed for the first 10 min with a linear gradient from 0
 to 35% (W/v) acetonitrile in 0.05% (w/v) TFA, which is
 followed by a shallow 35-36% gradient for the next 60 min.
 - Flow rate is adjusted to 0.8 ml/min; 1 min fractions are
 collected in Eppendorf tubes containing 10 ul of 1 M NH_4HCO_3
 15 and 5 ul of Tween 20 (1% (v/v) in water) and lyophilised.
 Total protein is measured fluorometrically with benzoxanthene
 following Neuheff et al. Hoppe-Seyler's Z. Physiol. Chem.
360:1657-1670, 1979. The purity of the final product is
 assessed by NaDodSO_4 /PAGE in 12% (w/v) acrylamide gels.
 20 Isoelectric focusing is performed with a LKB (Bromma, Sweden)
 vertical gel apparatus. Material is recovered from gels by
 overnight incubation in 130 mM NaCl containing Tween 20
 (10^{-4} v/v) and 10 mM sodium phosphate pH7.0. Affinity
 chromatography on lentil lectin-*Sepharose is done following
 25 the procedure described by the manufacturer (Pharmacia,
 -Uppsala, Sweden).

Amino Acid Sequence Analysis

Automated amino acid sequence analysis is performed
 with an Applied Biosystems sequencer (model 477A) equipped

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with an on-line phenylthiohydanthoin amino acid analyzer
1 (model 120A). In situ cyanogen bromide cleavage of P40
(~10 ug) is performed on the glass fiber sample disk of the
gas-phase sequencer according to a procedure described by
Simpson et al. Biochem. Internat. 8:787-791, 1984,
5 hereinafter Simpson I. Sequence comparisons are made with
the following databases: Protein Sequence database of PIR,
National Biomedical Research Foundation (release 15.0,
December 1987); Swiss-Prot Protein Sequence Data Bank version
5 (September 1987, compiled by A. Bairoch, University of
10 Geneva, Medical Biochemistry Department, 1211 Geneva 4,
Switzerland); G.B. trans Protein Data Base Release 1.0
(August 1987) compiled from GENBANK release 50.0 by J.
Coventry, Walter and Eliza Hall Institute of Medical
Research, Parkville 3050 Australia; and PG trans Protein Data
15 Base release 38.0 (December 1985) GENBANK, Instit. Pasteur,
Paris, France.

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EXAMPLE 2

1 Detection of T Cell Growth Factory Activity

TUC2.15 is a C57Bl/6 helper T cell line that requires antigen and antigen-presenting cells for long term
5 growth in vitro. In an attempt to grow these cells without feeders and antigens it is surprisingly discovered that, after supplementing the culture medium with 10% (v/v) autologous supernatant (SN) obtained after stimulation with ConA, this SN is able to induce cell proliferation without
10 further requirement for antigen or feeder cells. This growth factor activity is not inhibited by either anti-IL4 or anti-IL2 receptor antibodies (Table 1), indicating that the activity is mediated neither directly nor indirectly by these molecules.

15 In addition to its activity in short term proliferations, the SN also readily stimulates the development of permanent antigen-independent cell lines, which are maintained by subcultivation every 3-4 days in medium supplemented with 1% (v/v) SN (Fig. 1). Attempts to
20 derive antigen-independent cells lines with IL2 in this manner are to date unsuccessful. A second helper T cell clone, TUC7.51 also gives rise to an antigen-independent cell line upon culture in autologous SN. The factors active on the two cell lines are apparently identical, since TUC7.51 SN
25 supported the growth of TUC2.15 cells and vice versa.

TS1, one of the factor-dependent cell lines derived from TUC2.15 is selected for further identification of the growth factor. This choice is based on the observation that TS1 grows quickly, with a doubling time of 11 h, and responds
30 to very small concentrations of SN, half-maximal proliferation being obtained at dilutions between 10^{-5} and 10^{-4} (v/v). To determine the specificity of the TS1 assay, cells are incubated with a variety of purified growth factors or crude SN and found that only IL4 and TUC2.15 SN support
35 TS1 growth (Table 2). Since anti-IL4 antibodies fail to inhibit the effects of TUC2.15 SN, the aforementioned activity is a new T cell growth factor.

Proliferation of TUC2.15 Helper T Cells Induced By Autologous Supernatant (SN); Independence from IL2 and IL4

TUC2.15 helper T cells (5×10^4 /well) are incubated for 3 days with IL2 (100 U/ml), IL4 (100 U/ml) or TUC2.15 SN (1% v/v) in the presence of anti-IL2 receptor antibody 5A2 (30 ug/ml) or anti-IL4 antibody 11B11 (10 ug/ml). Thymidine incorporation is measured on day 3.

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TABLE 2

1 Growth of TS1 in Response to Various Cytokines

	<u>Factors</u>	<u>Dose/Dilution</u>	<u>Cell Growth (A^{405})</u>
5	TUC2.15 SN	1/12.500	1.96
	IL1	100 U/ml	0
	IL2	100 U/ml	0
	IL3	100 U/ml	0.01
	IL4	100 U/ml	1.36
10	IL5	100 U/ml	0
	IL6	20 ng/ml	0
	GM-CSF	10 ng/ml	0
	G-CSF	4 ng/ml	0
	M-CSF (crude)	1/4	0.02
15	EGF	50 ng/ml	0
	PDGF	4 ug/ml	0.02

20 TS1 cells are incubated for 3 days in the presence of various factors or SN. All reagents are tested over a 100-fold range but results are given for the highest dose only. None of the factors that score negatively at the highest dose have any effect at lower doses. Cell growth is measured by

25 colorimetric determination of hexosaminidase levels. Absorbance (A) of cultures at 405 nm incubated without growth factors ranges from about 0.10 to about 0.15 and is subtracted.

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EXAMPLE 3

1 Purification of the T Cell Growth Factor

Large batches of T cell SN are produced by stimulating TUC2.15 and TUC7.51 cells with ConA as described in Example 1. The active material is concentrated by adsorption to silicic acid and applied to an*Ultrogel AcA54 gel filtration column. The major growth promoting activity, which is destroyed by trypsin, elutes as a symmetrical peak in the 30-40 kDa region (Fig. 2A), and is therefore designated P40. Subsequent experiments are carried out with TUC7.51 SN because the concentrations of P40 are higher in this material.

-- Preliminary characterization of the growth factor indicates that it has a pI of ~ 10 and is glycosylated, 60% of the activity being retained on a lentil lectin column. Based in part on this information, the following purification protocol is adopted. Active fractions from the gel filtration step are further separated by hydrophobic interaction chromatography on a TSK-phenyl column (Fig. 2B) followed by passage through a*MonoQ anion exchange column equilibrated at pH 9.5. At this elevated pH, most contaminants are retained on the column, whereas P40 elutes mainly in the flow-through fractions, as expected from its high pI (Fig. 2C). Final purification is achieved by reversed phase chromatography on a C1-column equilibrated with 0.05% (w/v) TFA. P40 is recovered in a single peak eluting at an acetonitrile concentration of 35 % (v/v) (Fig. 2D). At the end of this purification, P40 stimulates half-maximal growth of TS1 at a concentration of ~ 5 pg/ml (Fig. 3), which corresponds to a 2000-fold purification. On average, the overall yield ranges from about 5 to about 10%.

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The purified protein is very heterogeneous with a
1 Mr of about 32 to about 39 kDa in NaDodSO₄/PAGE both under
reducing (Fig. 4) and non-reducing conditions. Biological
activity is recovered from the corresponding fractions of a
non-reduced gel, but exposure to NaDodSO₄ and
5 2-mercaptoethanol destroys most of the activity.

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EXAMPLE 4

1 Amino Acid Sequence Analysis of Murine P40

Edman degradation of P40 ($\approx$ 250 pmol) did not yield N-terminal sequence. For sequence analysis, P40
5 (immobilized on the polybrene-treated sample disk of the sequencer) is acylated (Tarr, Methods of Protein Micro-characterization [ed. J.E. Shively] Human Press, pp. 155-194, 1986) and then subjected to in situ cyanogen bromide treatment as described by Simpson I. Sequence analysis is
10 then continued and yields the following major amino acid sequence (110 pmol): NH_2 -Ala Gly Asn Thr Leu Ser Phe Leu Lys Ser Leu Leu Gly Thr Phe Gln Lys Thr Glu.

This internal sequence shows no significant similarity with that of other proteins stored in the data
15 bases listed in Example 1.

The determination of the complete amino acid sequence was achieved by chemical methods.

Briefly, before proteolytic digestion native P40 was reduced with dithiothreitol and carboxymethylated with
20 iodoacetic acid to yield Cm-P40. Peptides (indicated in Fig. 7) were prepared for sequence analysis by cleavage of Cm-P40 with endoproteinase Asp-N, trypsin, chymotrypsin, and cyanogen bromide (denoted by D, T, C, and N, respectively in Fig. 7). Subpeptides of the endoproteinase Asp-N were
25 derived by cleavage with Staphylococcus aureus V8 protease (denoted with hyphenated S suffixes). In Fig. 7, amino acid residues not identified are indicated by X.

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The exact methodology of amino acid sequence
1 determination is described below:

A. Materials: Tween 20, guanadine hydrochloride (Sequenal
grade) and trifluoroacetic acid (F_3AcOH ; 99 + % pure grade)
were purchased from Pierce Chemical Co. (Rockford, Illinois,
5 USA). Iodoacetic acid (puriss grade) was obtained from Fluka
(Buchs, Switzerland) and was recrystallized prior to use.
Dithiothreitol was from Calbiochem (La Jolla, California,
USA). Sodium chloride (Aristar grade) and acetic anhydride
were purchased from BDH (Poole, UK). Cyanogen bromide
10 (Univar grade) was from Ajax Chemical Co. (Sydney,
Australia). All other chemicals were of the highest grade
commercially available.

Trypsin (treated with tosylphenylethylchloro-
methane) and chymotrypsin were purchased from Worthington
15 Biochemical Co. (New Jersey, USA). Staphylococcus aureus
strain V8 protease was obtained from Miles Co. (Napperville,
Illinois, USA). Endoproteinase AspN from a Pseudomonas fragi
mutant and N-glycanase F were obtained from Boehringer
Mannheim GmbH (West Germany). All organic solvents were HPLC
20 grade (Chromar grade, Mallinckrodt, Kentucky, USA).
Deionized water, obtained from a tandem Milli-RO and Milli-Q
system (Millipore, Inc., Massachusetts, USA) was used for all
buffers.

B. Preparation of murine S-carboxymethyl-P40 (Cm-P40): P40
25 (15 ug in 120 ul 35% aqueous acetonitrile containing 0.1%
(v/v) F_3AcOH and 0.02% Tween 20 was concentrated to
approximately 10 ul by centrifugal centrifugation (Savant
Ind. Hicksville, NY), diluted to 160 ul with 7.5 M
guanidine.HCL containing 0.2 M Tris.HCl buffer, pH 8.5, 0.002
30 M EDTA and 0.02% (v/v) Tween 20 and then reduced with
dithiotreitol (0.015 M) at 40°C for 4.5 h. Alkylation was

achieved by the addition of iodoacetic acid (final
1 concentration, 0.05 M) to the mixture and incubation
continued for 30 min at 25°C in the dark. The reaction was
halted by addition of 25 ul of 2-mercaptoethanol. Cm-P40 was
recovered from the mixture using a reversed-phase
5 high-performance liquid chromatography (RP-HPLC) procedure
previously described (Simpson et al., Eur. J. Biochem.
1176:187-197, 1988 hereinafter Simpson II). The Cm-P40-
containing fraction (60 ul) was adjusted to 0.02% (v/v) with
respect to Tween 20 and then diluted to 1 ml with an
10 appropriate buffer (containing 0.02% (v/v) Tween 20) prior to
enzymatic digestion.

C. Trypsin digestion: Cm-P40 (7 ug) in 1 ml of 1% (w/v)
NH₄HCO₃, pH 7.8 containing 0.001 M CaCl₂ and 0.02% (v/v)
Tween 20 was digested with 0.5 ug trypsin for 16 h at 37°C.

15 D. Chymotrypsin digestion: Cm-P40 (6 ug) in 1 ml of 1%
(w/v) NH₄HCO₃, pH 7.8 and 0.02% (v/v) Tween 20 was digested
with 0.6 ug chymotrypsin for 16 h at 37°C.

E. Endoproteinase Asp-N digestion: Cm-P40 (15 ug) in 1020
ul of 0.05 M sodium phosphate buffer, pH 8.1, 0.02% (v/v)
20 Tween 20 was digested with 0.7 ug of freshly prepared
endoproteinase Asp-N for 16 h at 34°C.

F. Staphylococcus aureus strain V8 protease digestion:
Endoproteinase Asp-N peptide D3 in 1 ml of 1% (w/v) NH₄HCO₃,
0.02% (v/v) Tween 20 was digested with 0.5 ug S. aureus V8
25 protease at an enzyme/substrate ratio of 1:10 for 16 h at
30°C.

G. Purification of polypeptides by high-performance liquid
chromatography instrumentation: Peptide mixtures resulting
from enzymatic cleavages were fractionated by reversed-phase
30 HPLC on a Hewlett-Packard liquid chromatograph (model 1090A)
fitted with a diode-array detector (model 1040A) as described
(Simpson II).

H. Column Supports: The following columns were used for the purification of Cm-P40 and derived peptides: (a) Brownlee RP-300 (300nm pore size, 7-um particle diameter, octylsilica packed into a stainless steel column 30 x 2.1 mm i.d. or 50 x 1 mm i.d., Brownlee Laboratories, Santa Clara, California, USA). (b) Dimethylaminoazobenzene sulfonyl chloride (DABS-Cl) amino acids were separated and quantitated on a Brownlee PTC amino acid analysis column (220 x 2.0 mm i.d.) (Applied Biosystems, Foster City, California, USA).

I. Peptide nomenclature: The following prefixes are used to denote the origin of various peptides: T, Trypsin; CN, cyanogen bromide; C, chymotrypsin; D, endoproteinase Asp-N. Peptides resulting from sub-digestion of endoproteinase Asp-N peptides with S. aureus V8 protease are denoted by hyphenated S suffixes. Peptides are numbered in the order of their positions in the final sequence.

J. Cyanogen bromide cleavage: After P40 (10 ug) was subjected to several cycles of Edman degradation in the protein sequencer without any detectable PTH-amino acids, the sequence analysis was stopped. In situ cyanogen bromide cleavage of native P40 was performed on the glass fiber sample disk of the protein sequencer according to a procedure previously described (Simpson, I). Sequencer background levels which had arisen during the sequence analysis were reduced by treating the sample disk with 30 ul aqueous 50% (v/v) N-ethylmorpholine followed by 10 ul acetic anhydride (60 min at 25°C). The filter was vacuum dried and then treated with a 20-fold excess of cyanogen bromide in 70% (v/v) formic acid for 15 h at 25°C. At the end of this time the sample filter was vacuum dried for 30 min and the sequence analysis continued.

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K. Amino acid sequence analysis: Automated Edman

1 degradation of protein and peptide was performed using
Applied Biosystems sequencers (models 470A and 477A) equipped
with on-line phenylthiohydantoin (Pth) amino acid analyzers
(model 120A). Total Pth-amino acid derivatives from the
5 sequencer were injected onto the liquid chromatograph using a
modified sample transfer device as described [Begg et al., in
"Techniques in Protein Chemistry", (Hugli, T.E., Ed.)
Academic Press, Orlando Fl., USA, 1989]. Polybrene was
used as a carrier.

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EXAMPLE 5

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Peptide purification by microbore RP-HPLC

Cm-P40 (15 ug) was digested with endoproteinase Asp-N and the digest fractionated by RP-HPLC on a short
5 microbore column (30 x 2.1 mm i.d.), employing a low-pH (F₃ AcOH, pH 2.1) mobile phase and a gradient of acetonitrile. Three major peptide-containing peaks were detected: D1, D2 and D3 (Fig. 8). Spectral analyses of these peptides were performed using real-time photodiode-array spectroscopy and
10 the absorption spectra of peptides D1, D2 and D3 are shown in Fig. 9. The high absorbance at 290 nm of peptide D1 is indicative of the presence of a tryptophan residue. The D2 and D3 peptides have high absorbance at 280 nm and low absorbance at 290 nm which is characteristic of tyrosine-
15 containing peptides. The presence of tryptophan residue in peptide D1 is supported by the derivative absorbance spectrum shown in Fig. 10. Enhancement of resolution by second-order-derivative spectroscopy reveals extrema at 290; 2 nm and 280 ± 2 nm which are characteristic of tryptophan residues.
20 Peptide D3 was subdigested with S. aureus V8 protease and the resultant digest fractionated by RP-HPLC at low pH (F₃AcOH) (Fig. 11A).

Reversed-phase HPLC purification of peptides resulting from treatment of Cm-P40 with chymotrypsin and
25 trypsin were performed and analyzed in a similar manner. Reversed-phase fractionation of these digests, however, resulted in a complex pattern of peptide-containing peaks (Figs. 11B and 11C). All of the peptide fractions from the first dimension RP-HPLC were subjected to a second
30 chromatographic step using the same chromatographic support and acetonitrile gradient but a different mobile phase (e.g.,

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unbuffered sodium chloride or 20 mM sodium phosphate, pH
1 7.0). For some peptides a third chromatographic step was
necessary before a homogeneous peptide could be isolated. In
the latter situation, an *ODS-hypersil column and a different
organic solvent (methanol) were used for the chromatography
5 (Simpson II).

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EXAMPLE 6

1 Characterization of glycosylation state of P40

 Cm-P40 (0.5 ug) was iodinated using the iodine
monochloride procedure. ¹²⁵I-Cm-P40 was separated from free
5 ¹²⁵I by sequential gel filtration and cation-exchange
chromatography. ¹²⁵I-Cm-P40, untreated, reduced with
2-mercaptoethanol for 5 min at 95°C, or digested with
N-glycanase F (Genzyme, Boston, MA, USA) or endo- α -N-
acetylgalactosaminidase (O-glycan-peptide hydrolase,
10 Boehringer Mannheim) for 16 h at 37°C according to the
manufacturers' instructions was electrophoresed on a 10-15%
gradient polyacrylamide gel in the presence of SDS. The gel
was stained with Coomassie Blue R250 using the Phast
electrophoresis sytem (Pharmacia, Uppsala, Sweden) according
15 to the manufacturer's instructions. ¹²⁵I-Cm-P40 was detected
by autoradiography using Hyperfilm, MP (Amersham,
Buckinghamshire, UK).

 Purified iodinated P40 electrophoresed as a single
broad band of apparent M_r 32,000-39,000 daltons on 12%
20 SDS-PAGE in both the unreduced and reduced
(2-mercaptoethanol) state (see Fig. 4, Lane 1 and 4)
indicating that it is a monomeric protein. Endo- α -N-
acetylgalactosaminidase (O-glycanase) treatement of P40 had
no apparent effect on the molecular mass (Fig. 4, Lane 3) but
25 treatment with N-glycanase F caused a reduction in apparent
 M_r to 15,000-16,000 Da (Fig. 4, Lane 2). The lack of effect
of O-glycanase indicates that P40 does not contain O-linked
carbohydrate chains or that these sites are not accessible in
the intact molecule. Since N-glycanase F releases
30 carbohydrate moieties attached to asparagine residues
(N-linked) this indicates that P40 consists of a protein core
(M_r 15,000-16,000) with considerable amounts of N-linked
sugars.

Murine P40 has 126 amino acids. The calculated M_r from the sequence analysis is 14,150. The difference in the calculated M_r and the measured M_r for native P40 (32-39 kDa) can be attributed to N-glycosylation since upon treatment with N-glycanase F the M_r is reduced to 15,000-16,000 (Fig. 4). The protein sequence data provides information on the post-translational processing of mature P40. For instance, since no amino acid was identified at positions 32, 60, 83 and 96 (Fig. 10) and since these positions meet the criteria for N-linked glycosylation sites (i.e. Asn-Xaa-Thr/Ser), these data are consistent with asparagines being glycosylated at these four positions. Confirmation of asparagine residues at positions 32, 60, 83 and 96 and the COOH - terminal residue (Pro-126) was provided by sequence analysis of a P40 cDNA clone.

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EXAMPLE 7

1 Analysis of N-terminal block in murine P40

The exact nature of the blocked N-terminus was determined by a combination of amino acid analysis, 5 fast-atom-bombardment mass spectrometry, and peptide synthesis. These analyses indicated the N-terminus of murine P40 is likely to be pyroglutamic acid.

The methods involved in determining the amino terminal residue of P40 and a discussion thereof follow:

10 Method

A. Amino acid analysis: Amino acid analysis was performed on a Beckman amino acid analyzer (model 6300) equipped with a model 7000 data system or by using the dimethylaminoazobenzene sulfonyl chloride (DABS-Cl) precolumn derivatization 15 procedure using microbore column RP-HPLC (Simpson, et al., Euro. J. Biochem. 153: 629-637, 1985). Samples were hydrolyzed in vacuo at 110°C for 24 h with gaseous HCl generated from 6M HCl containing 0.1% (w/v) phenol.

B. Fast-atom-bombardment mass spectrometry: Fast-atom- 20 bombardment mass spectrometry (Barber et al., Anal. Chem., 54: 645A-657A, 1982) was performed using a VG 70/70E-HF forward-geometry double-focussing mass spectrometer (VG Analytical, Manchester, UK) equipped with an Ion Tech saddle-field fast-atom gun. Sample was applied in 2 ul of 0.1% 25 (v/v) aqueous F₃AcOH to the sample stage containing 1 ul of pre-applied mixture of dithiothreitol/dithioerythritol (5:1). Xenon atoms at a potential of 8keV and a discharge potential of 1 mA were used for sample bombardment. Scans were performed at 40s/decade at a resolution of 1500.

30 Positive-ion spectra were acquired by multi-channel analysis

mode using a VG 11/250J data system.

1 C. Peptide synthesis: Fluorenylmethoxycarbonyl (Fmoc)-
polyamide solid phase peptide chemistry was employed to
synthesize two peptides corresponding to the N-terminal
decapeptide of P40 (endoproteinase Asp-N peptide D1) with
5 either glutamic acid or glutamine as the amino terminal
residue. The conventional Fmoc polyamide side chain
protecting groups were employed: Trp (CHO); Arg (Mtr), Mtr =
4-methoxy-2,3,6,-trimethylbenzenesulfonyl); Glu (OtBu); Cys
(tBu). Pentafluorophenyl (OPfp) esters of all Fmoc-amino
10 acids in dimethylformamide with the exception of Fmoc-Arg
(MtC) which was activated as an 1-hydroxybenzotriazole (HOBt)
ester, were employed for sequential coupling of activated
amino acids on *RaMPS (DuPont, NJ) Wang resin. Peptide bond
formation was generally complete within 120 min provided that
15 the concentration of the OPfp ester was 2.5 x times greater
than the concentration of derivatized resin and provided that
one equivalent of 1-hydroxybenzotriazole was added to the
coupling mixture in order to catalyze the reaction. Upon
completion of the synthesis, the peptide was deprotected and
20 cleaved from the resin by extended treatment with F₃AcOH
(containing 5.4% thioanisol, 0.6% 1,2-ethanedithiol). Crude
synthetic peptides and their derivatives (e.g.
S-carboxymethyl-peptides) were purified by reversed-phase
HPLC. The pure synthetic peptides chromatographed as single
25 peaks on reversed-phase HPLC (Brownlee RP-300 column 30 x 2.1
mm, i.d.) and gave the expected amino acid ratios.

Peptides (30 ug in 100 ul 0.1% F₃AcOH) were
acetylated with acetic anhydride by treatment with 6 ul

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N-ethylmorpholine (Pierce, sequenal grade) followed by 2 ul
1 acetic anhydride (Fluka, puriss grade) for 10 min at 25°C.
Formation of pyroglutamyl peptides was accomplished by
treating the glutamine peptide at 110°C for 16 h at pH 7.8
under nitrogen.

5 Discussion

Analysis of three major Asp-N peptides (D1-D3) and
the S. aureus V8 protease subpeptides of D3 provided 65% of
the P40 amino acid sequence. Of the three peptides, D1, the
single tryptophan-containing peptide was N^α blocked,
10 indicating that this peptide was derived from the N-terminal
portion of the polypeptide chain. The amino acid composition
of the N-blocked Asp-N peptide D1 was consistent with the
tryptophan-containing tryptic peptide T1 with the N-terminal
addition of two extra residues (Glx and Arg).

15 Fast-atom-bombardment mass spectrometry (FAB-MS) of
Asp-N peptide D1 revealed a protonated molecular ion (MH⁺) of
mass 1248 which only corresponds to the amino acid
composition of this peptide if the blocking group was assumed
to be pyroglutamic acid. The nature of the N-blocking group
20 was examined using two synthetic decapeptides D1 with either
glutamic acid or glutamine at the amino terminus (see Fig. 7
and 12). As shown in Fig. 12, the endoproteinase Asp-N
peptide D1 and the pyroglutamyl synthetic peptide
co-chromatographed on reversed-phase HPLC (retention time,
25 20.50 ± .2 min) and were well resolved from the glutamyl
synthetic peptide (retention time, 22.70 ± 0.2 min). After
acetylation, the chromatographic behavior of D1 and the
pyroglutamyl synthetic peptide was identical (no increase in
retention time) while the acetylated glutamyl synthetic
30 peptide exhibited a marked increase in retention behavior

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1 (retention time was 28.97 ± 0.2 min compared to the
non-acetylated form, 22.70 ± 0.2 min). The amino terminus of
P40 is likely to be pyroglutamic acid since the amino-
terminal endoproteinase Asp-N peptide D1 behaves in exactly
5 the same manner as the synthetic pyroglutamyl peptide before
and after acetylation on reversed-phase HPLC. FAB-MS of the
glutamyl synthetic peptide yielded a molecular mass of 1248
which was in perfect agreement with that obtained for Asp-N
peptide D1.

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EXAMPLE 8

1 Biological Activity of Purified P40

 Purified P40, at concentrations up to 20 ng/ml, did
not support the proliferation of either IL3-dependent myeloid
5 cell lines (FDCP-1, Ea3.15 and DA-1), IL5-dependent B cell
lymphoma BCL1, or IL6-dependent B cell hybridoma 7TD1.
Unlike IL2, and to some extent IL4, it also fails to
stimulate any of six cytolytic T cell clones tested (Table
3). By contrast, strong proliferations are observed with
10 some but not all helper T cell lines. Both IL2-producing
(TH₁ type, TUC7.33) and IL4-producing (TH₂ type, e.g.,
TUC2.15) clones are found among the responders. A
significant correlation, illustrated in Table 3 for clone
TUC7.51, is observed between the time spent in culture and
15 the responses to P40 and IL4.

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TABLE 3

Comparison of the T Cell Growth Factor Activities of IL2, IL4, and Purified P40

T Cell Lines and Clones

1 3 4 1 4 6 1

IL2

Proliferation in response to^a

IL4

P40

Cytolytic T Cells

CTL-2

445

0.6

P35:10

683

24

P35:48

303

4

P91:6

195

2

P1:5

993

12

P1:204-8

630

9

Helper T Cells

TUC2

311

2

TUC2.15

1,806

813

TUC5

263

20

TUC5.37

253

4

TUC7.33

115

276

TUC7.51^b

1,179

27

TUC7.51^c

1,345

634

TUC13.1

116

51

1.7

A) Cells are incubated with or without the indicated growth factors and thymidien incorporation is measured on day 3. Factor dosage is as follows: 100 U/ml for IL2 and IL4, and 10^3-10^4 U/m for P40. The results are shown as ratios of radioactivity incorporated with and without factors.

B) 1 month-old culture

C) 1 year-old culture

EXAMPLE 9

Cloning and Characterization of the Murine P40 Gene

Screening of cDNA library. Double-stranded cDNA was prepared according to Gubler, et al., Gene 25: 263, 1983, using polyadenylated RNA isolated from P40-producing helper T cells TUC7.51 after a 24 h stimulation with ConA (2.5 ug/ml). The cDNA was cloned into the BamHI site of a pUC8 vector and transformed into E. Coli strain DH5. Transformants were screened by in situ hybridization with two end-labeled 20-mer oligonucleotide probes. For the initial screening, a 64-fold degenerate probe

[5'-TGCAT(C+T)TC(X)GT(C+T)TT(C+T)TG(G+A)AA-3'] corresponding to amino acid sequence FQKTEMQ (positions 114-120, see text) was used. Positive clones were subsequently tested with a

129-fold degenerate probe

[5'-GG(A+G)TC(A+G)TC(T+C)TT(X)AG(A+G)TT(C+T)TC-3'] corresponding to sequence ENLKDDP (positions 17-23, see text).

DNA sequencing. DNA was sequenced by the dideoxynucleotide procedure after subcloning into a M13 vector. Appropriate fragments were generated by digestion with PstI and NcoI restriction endonucleases using the sequencing strategy shown in Fig. 5.

Characterization of cDNA and Construction of Expression Vectors. A cDNA library was prepared, in a pUC8 vector, from a helper T cell clone that produces large amounts of P40 after stimulation with ConA. This library was screened with two oligonucleotide probes synthesized on the basis of selected amino acid sequence data obtained by

analysis of P40 peptides. Of 20,000 independent
 1 transformants, 112 hybridized with the two probes. Most of
 these clones contained cDNA inserts of about 500bp. Using
 one of these cDNA's as a probe, a strong signal was obtained
 with a transcript of about 700 nucleotides in Northern blots
 5 of poly(A)⁺RNA isolated from P40-producing helper T cell
 clone TUC7.51. Poly(A)⁺RNA from P815 mastocytoma, which does
 not produce detectable P40 activity, gave no signal at all.

To establish that the selected clones contained
 authentic P40 cDNA, an expression vector was constructed.

10 Insert P40.2B4 was cloned into BamHI site of plasmid
 pZIPneoSV(X)1 (Cepko, et al., Cell 37: 1053, 1984) and
 transfected into Clone-1d fibroblasts (Kit, et al., Exp.
Cell. Res. 31: 297, 1963). Cell supernatants collected 48 h
 after transfection were tested for their growth factor
 15 activity on P40-dependent TS1 cells. As shown in Fig. 6,
 supernatants from cells transfected with P40.2B4 cDNA (closed
 symbols), but not from mock-transfected cells (open symbols),
 supported the growth of TS1. This result indicates that
 P40.2B4 cDNA presumably contains the entire coding region of
 20 P40.

The complete nucleotide sequence of the cDNA insert
 of clone P40.2B4 was determined, and is shown under "Detailed
 Description of the Invention". It consists of 554
 nucleotides with a 5' untranslated sequence of 15
 25 nucleotides, an open reading frame of 432 nucleotides and a
 3' untranslated region of 107 nucleotides. The 3'-end
 terminates with a string of 18 adenine residues located 12
 nucleotides downstream from an AATAAA polyadenylation signal
 consensus sequence. The 3'untranslated region contains 3

copies of the sequence ATTTA which is characteristic of
 1 transiently expressed genes such as GM-CSF, G-CSF,
 interferons, several interleukins, tumor necrosis factor, and
 oncogenes c-fos and c-myc (Shaw et al., Cell 46: 659, 1986);
 two of these repeats at nucleotide positions 461-468 and
 5 470-477 are part of an 8 nucleotide motif TATTTATT, which is
 also present in many of these molecules (Caput et al., Proc.
Natl. Acad. Sci. USA 83: 1670, 1986).

The predicted polypeptide encoded by the cDNA
 insert of clone P40.2B4 consists of 144 residues. This size
 10 estimation is based on the presumption that the first ATG in
 the sequence (nucleotide position 16-18) is the initiator
 codon, a view supported by the efficient expression of the
 cDNA in fibroblasts and by the presence of an adenine at
 nucleotide position 13, in concordance with the consensus
 15 sequence for an initiator ATG codon; an in-frame TGA
 translation termination codon occurs as nucleotides 448-450.
 The deduced P40 sequence is characterized by a hydrophobic
 N-terminal sequence typical of a signal peptide. Because of
 the presence of a blocked N-terminus in the native protein,
 20 there is some uncertainty concerning the N-terminal residue.
 Based on the probability weight-matrix described, (Von
 Heijne, Nucleic Acid Res. 14: 4683, 1986), the most likely
 N-terminal sequence of the mature protein would be
 Gln-Arg-Cys... This is consistent with evidence obtained by
 25 biochemical analysis of P40 peptides. Mature P40 would then
 consist of 126 amino acids with a predicted relative
 molecular mass of 14,150. The difference with the Mr
 measured for native P40 appears to be due to glycosylation as
 suggested by the presence of 4 potential N-linked

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1 3 4 1 4 6 1

glycosylation sites and confirmed by the about 15kDa Mr of
1 the native protein after N-glycanase-treatment. The sequence
of P40 is further characterized by the presence of 40
cysteines and a strong predominance by cationic residues,
which explains the elevated pI(10) of the native protein.

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EXAMPLE 10

1 Isolation of the Human Genomic P40 Gene

5 The human genomic P40 gene was cloned using the
murine a P40 cDNA clone as a probe. Briefly, a human genomic
library was constructed in phage λ GEMII with Sau3A-cut DNA
isolated from an EBV-immortalized lymphoblastoid cell line
(CESS). A resulting clone (λ H403A1) was isolated by cross-
hybridization with the murine P40 cDNA.

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1 3 4 1 4 6 1

EXAMPLE 11

1 Isolation of the Human cDNA for P40

5 A cDNA library was constructed with poly-A⁺RNA
prepared from peripheral blood mononuclear cells stimulated
for 24 h with phytohemagglutinin and phorbol myristate
acetate. The library was screened with a restriction
fragment of the human genomic clone described in Example 10.
One of the positive clones was sequenced by the methods
described in Example 10, and the DNA sequence with its
10 deduced amino acid sequence is shown under the "Detailed
Description of the Invention". Sequence analysis indicated
that the murine and human P40 amino acid sequences are 55%
homologous.

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EXAMPLE 12

1 Synergistic Effect of P40 and IL4 or IL3

 The proliferative effects of co-culturing helper T
cells in the presence of P40 and IL4 or IL3 was investigated.
5 TUC2.15N and TUC7.41 cells (5×10^4 /well) were cultured with
suboptimal amounts of P40 in the presence or absence of a
suboptimal dose of IL4 or close to optimal dose of IL3.
After 3 days in culture, the cells were pulsed with tritiated
thymidine. The results in Table 4 indicate that the helper T
10 cells treated with P40 and IL4 or P40 and IL3 incorporated
from about 4-40 times more thymidine than those cells treated
with any one of these proteins. Thus, a strong synergy
between P40 and IL4 or IL3 exists with respect to stimulating
proliferation of helper T cell lines that respond to these
15 proteins.

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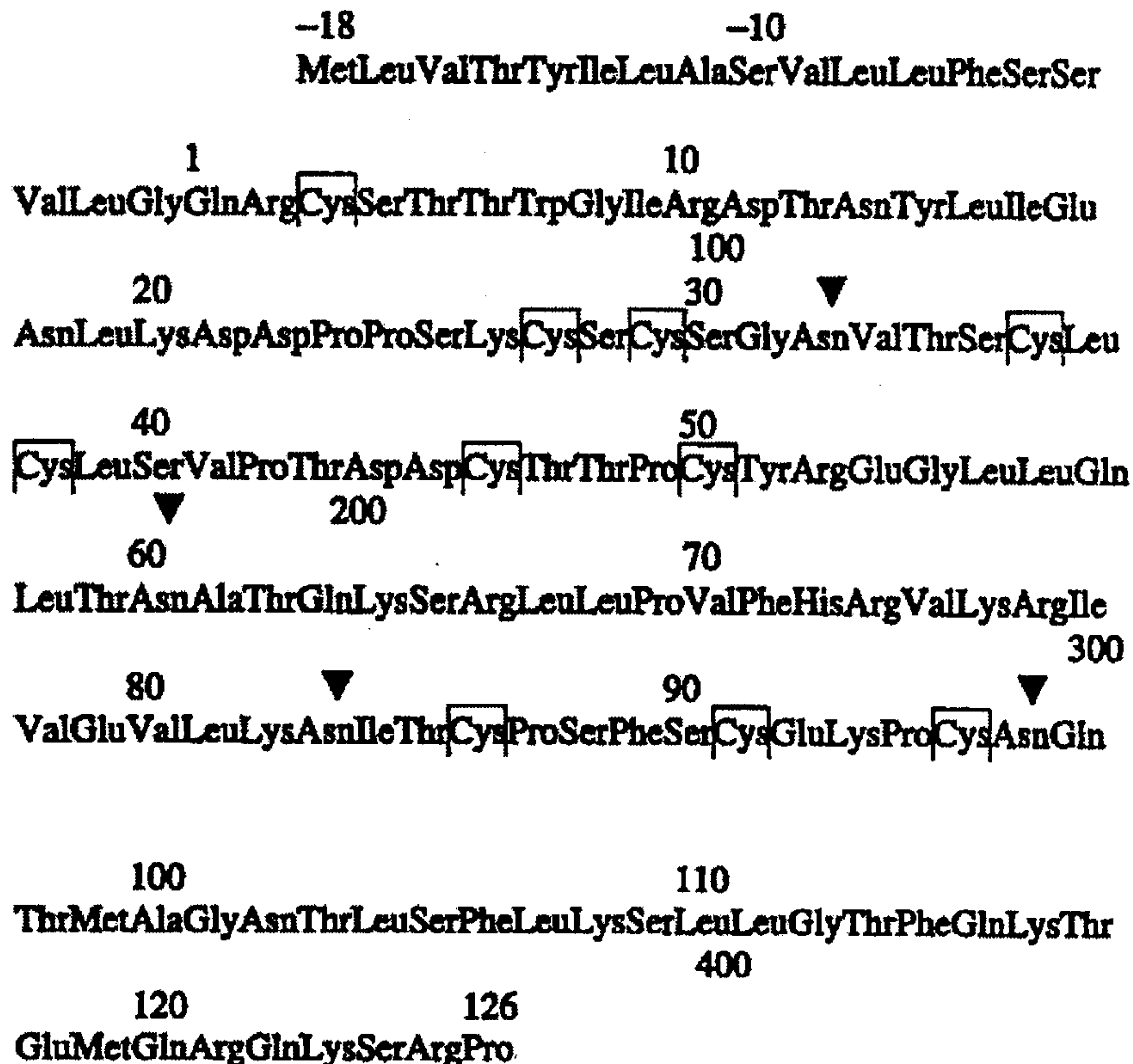
TABLE 4
Synergism between P40 and IL4 or IL3

Cells	P40 (U/ml)	IL4 (U/ml)	IL3 (ng/ml)	Thymidine Incorporation (cpm)
TUC2.15N	0	0	0	122
	80	0	0	1296
	0	20	0	4446
	80	20	0	19248
	0	0	3	4781
	80	0	3	54366
TUC7.51N	0	0	0	120
	80	0	0	4958
	0	20	0	4459
	80	20	0	31050
	0	0	3	4354
	80	0	3	72157

TUC2.15N and TUC7.51 helper T cells (5×10^4 /well) were cultured with suboptimal amounts of P40 in the presence or absence of a suboptimal dose of IL4 or of an optimal dose of IL3. The cultures were pulsed with tritiated thymidine after 3 days.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An isolated biologically pure, mammalian T cell growth factor molecule which is a single chain glycoprotein having a molecular weight of from about 30 kDa to about 40 kDa and which supports interleukin-2 and interleukin-4 independent growth of helper T cells, wherein said single chain glycoprotein is P40.
2. The T cell growth factor of claim 1 wherein mammalian is murine or human.
3. The T cell growth factor of claim 1 wherein said glycoprotein has a protein moiety having a molecular weight of about 14 kDa to about 16 kDa.
4. The T cell growth factor of claim 1 wherein said glycoprotein is murine P40 having an amino acid sequence which comprises:



1 3 4 1 4 6 1

5. The T cell growth factor of claim 1, wherein said P40 is murine P40 having an amino acid sequence which comprises:

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      1                                10
      GlnArgCysSerThrThrTrpGlyIleArgAspThrAsnTyrLeuIleGlu
                                100
      20                                30
      AsnLeuLysAspAspProProSerLysCysSerCysSerGlyAsnValThrSerCysLeu
                                ▼
      40                                50
      CysLeuSerValProThrAspAspCysThrThrProCysTyrArgGluGlyLeuLeuGln
      ▼                                200
      60                                70
      LeuThrAsnAlaThrGlnLysSerArgLeuLeuProValPheHisArgValLysArgIle
                                300
      80                                90
      ValGluValLeuLysAsnIleThrCysProSerPheSerCysGluLysProCysAsnGln
                                ▼
      100                                110
      ThrMetAlaGlyAsnThrLeuSerPheLeuLysSerLeuLeuGlyThrPheGlnLysThr
                                400
      120                                126
      GluMetGlnArgGlnLysSerArgPro
  
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6. The T cell growth factor of claim 1, wherein said P40 is human P40 having an amino acid sequence which comprises:

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      -18
      MET LEU LEU ALA MET VAL LEU THR SER ALA LEU LEU LEU CYS SER VAL ALA
      1
      GLY GLN GLY CYS PRO THR LEU ALA GLY ILE
      10
      LEU ASP ILE ASN PHE LEU ILE ASN LYS MET
      20
      GLN GLU ASP PRO ALA SER LYS CYS HIS CYS SER ALA ASN VAL THR SER CYS LEU CYS LEU
      30
      40
      GLY ILE PRO SER ASP ASN CYS THR ARG PRO CYS PHE SER GLU ARG LEU SER GLN MET THR
      50
      60
      ASN THR THR MET GLN THR ARG TYR PRO LEU ILE PHE SER ARG VAL LYS LYS SER VAL GLU
      70
      80
      VAL LEU LYS ASN ASN LYS CYS PRO TYR PHE SER CYS GLU GLN PRO CYS ASN GLN THR THR
      90
      100
      ALA GLY ASN ALA LEU THR PHE LEU LYS SER LEU LEU GLU ILE PHE GLN LYS GLU LYS MET
      110
      126
      ARG GLY MET ARG GLY LYS ILE
  
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7. The T cell growth factor of claim 1, wherein said P40 is human P40 having an amino acid sequence which comprises:

1	GLN	GLY	CYS	PRO	THR	LEU	ALA	GLY	ILE	10	LEU	ASP	ILE	ASN	PHE	LEU	ILE	ASN	LYS	MET	
20	GLN	GLU	ASP	PRO	ALA	SER	LYS	CYS	HIS	CYS	30	SER	ALA	ASN	VAL	THR	SER	CYS	LEU	CYS	LEU
40	GLY	ILE	PRO	SER	ASP	ASN	CYS	THR	ARG	PRO	50	CYS	PHE	SER	GLU	ARG	LEU	SER	GLN	MET	THR
60	ASN	THR	THR	MET	GLN	THR	ARG	TYR	PRO	LEU	70	ILE	PHE	SER	ARG	VAL	LYS	LYS	SER	VAL	GLU
80	VAL	LEU	LYS	ASN	ASN	LYS	CYS	PRO	TYR	PHE	90	SER	CYS	GLU	GLN	PRO	CYS	ASN	GLN	THR	THR
100	ALA	GLY	ASN	ALA	LEU	THR	PHE	LEU	LYS	SER	110	LEU	LEU	GLU	ILE	PHE	GLN	LYS	GLU	LYS	MET
	ARG	GLY	MET	ARG	GLY	LYS	126	ILE													

8. The T cell growth factor of claim 1 wherein said protein is capable of augmenting proliferation of IL3 or IL4-responsive cells.
9. An active derivative or an active fragment of human or murine P40 wherein said activity is stimulating interleukin 2- and interleukin 4-independent growth of helper T cells.
10. An isolated proteolytic fragment of human or murine P40 suitable for use in diagnostic assays for P40.
11. A method of preparing a mammalian T cell growth factor which is a single chain glycoprotein having a molecular weight of from about 30 kDa to about 40 kDa capable of supporting interleukin 2- and interleukin 4- independent growth of helper T cells which comprises contacting a helper T cell culture with an antigen or a mitogen for a time and under conditions sufficient to

X

induce production of said factor and recovering said factor from said culture.

12. A method of purifying human or murine P40 which comprises contacting a culture supernatant of an antigen- or mitogen- stimulated helper T cell line with a hydrophobic chromatography resin, identifying and recovering a first fraction therefrom having P40 activity, contacting said first fraction with an ion exchange chromatography resin, identifying and recovering a second fraction therefrom having said activity, contacting said second fraction with a reverse-phase HPLC resin and identifying and recovering a third fraction therefrom having said activity whereby said third fraction is homogeneous P40.
 13. The method of claim 12 wherein said hydrophobic resin is *TSK-phenyl chromatography resin, said ion exchange resin is *Mono-Q chromatography resin and said reverse-phase resin is C1 reversed-phase HPLC resin.
 14. The method of claim 12 wherein said activity is stimulating interleukin 2- and interleukin 4-independent growth of helper T cells.
 15. Use of human or murine P40, an active derivative thereof, or an active fragment thereof in an amount effective to stimulate proliferation of helper T cells in a mammal.
 16. Use of human or murine IL3 and P40 or an active derivative or fragment thereof in an amount effective to stimulate proliferation of IL3-responsive cells in a mammal.
 17. Use of human or murine IL4 and P40 or an active derivative or fragment thereof in an amount effective to stimulate proliferation of IL4-responsive cells in a mammal.
 18. The use as defined in claim 16 or 17, wherein said cells are helper T cells.
- *Trade-mark

19. The use as defined in claim 15, 16 or 17, wherein said mammal is a human.
20. The use as defined in claim 15, 16 or 17, wherein the effective amount of said P40, said P40 derivative, said P40 fragment, said IL3 or said IL4 is from about 10 µg to about 1000 µg per kilogram of body weight per day.
21. The use as defined in claim 15, 16 or 17, wherein the use is intravenous, intramuscular, intranasal, intradermal, intraperitoneal, suppository or oral.
22. The use as defined in claim 15, wherein a nucleic acid molecule encoding P40, an active derivative thereof, or an active fragment thereof is utilized.
23. The use as defined in claim 16, wherein a nucleic acid encoding IL3 or P40 is utilized.
24. The use as defined in claim 17, wherein a nucleic acid encoding IL4 or P40 is utilized.
25. Use of human or murine P40, an active derivative thereof, or an active fragment thereof in an amount effective to stimulate proliferation of helper T cells and treat immune deficiency in a mammal.
26. Use of IL3 and human or murine P40 or an active derivative or fragment thereof in an amount effective to stimulate proliferation of IL3-responsive cells and treat immune deficiency in a mammal.
27. Use of IL4 and human or murine P40 or an active derivative or fragment thereof in an amount effective to stimulate proliferation of IL4-responsive cells and treat immune deficiency in a mammal.
28. The use as defined in claim 26 or 27 wherein said cells are helper T cells.
29. The use as defined in claim 25, 26 or 27 wherein said mammal is a human.

30. The use as defined in claim 25, 26 or 27 wherein the effective amount of said P40, said P40 derivative or said P40 fragment, said IL3 or said IL4 is from about 10 μ g to about 1000 μ g per kilogram of body weight per day.
31. The use as defined in claim 25, 26 or 27 wherein the use is intravenous, intramuscular, intranasal, intradermal, intraperitoneal, suppository or oral.
32. A pharmaceutical composition for inducing proliferation of helper T cells in a mammal comprising an effective amount of human or murine P40, an active derivative thereof, an active fragment thereof, or a nucleic acid molecule which encodes human or murine P40, an active derivative thereof, or an active fragment thereof, and a pharmaceutically acceptable carrier.
33. A pharmaceutical composition for inducing proliferation of IL3-responsive cells in a mammal comprising an effective amount of IL3 and human or murine P40, or a nucleic acid molecule which encodes IL3 and human or murine P40, and a pharmaceutically acceptable carrier.
34. A pharmaceutical composition for inducing proliferation of IL4-responsive cells in a mammal comprising an effective amount of IL4 and human or murine P40, or a nucleic acid molecule which encodes IL4 and human or murine P40, and a pharmaceutically acceptable carrier.
35. The pharmaceutical composition of claim 32, wherein the effective amount of said P40, said P40 derivative, or said P40 fragment is from about 10 μ g to about 1000 μ g per kilogram of body weight per day.
36. The pharmaceutical composition of claim 33, wherein the effective amount of each of said IL3 and human or murine P40 is from about 10 μ g to about 1000 μ g per kilogram of body weight per day.

37. The pharmaceutical composition of claim 34, wherein the effective amount of each of said IL4 and human or murine P40 is from about 10 μ g to about 1000 μ g per kilogram of body weight per day.
38. An antibody to the T cell growth factor of claim 1.
39. The antibody of claim 38 wherein mammalian P40 is murine P40 or human P40.
40. An antibody to mammalian P40, an antigenic derivative thereof or an antigenic fragment thereof.
41. The antibody of claim 40 wherein said antibody is polyclonal.
42. The antibody of claim 41, wherein mammalian P40 is murine P40 or human P40.
43. A method for detecting P40 in mammalian tissue, tissue extract or serum which comprises contacting said tissue, tissue extract or said serum with an antibody of claim 38 for a time sufficient for a P40 antibody complex to form and subjecting said P40 antibody complex to a detecting means.
44. A method for detecting P40 in mammalian tissue, tissue extract or serum which comprises contacting said tissue, tissue extract or said serum with an antibody of claim 40 for a time sufficient for a P40 antibody complex to form and subjecting said P40 antibody complex to a detecting means.
45. An isolated nucleic acid encoding human or murine P40 or a derivative thereof.
46. The nucleic acid of claim 45 wherein said nucleic acid is an RNA, mRNA, a cDNA or a recombinant DNA.



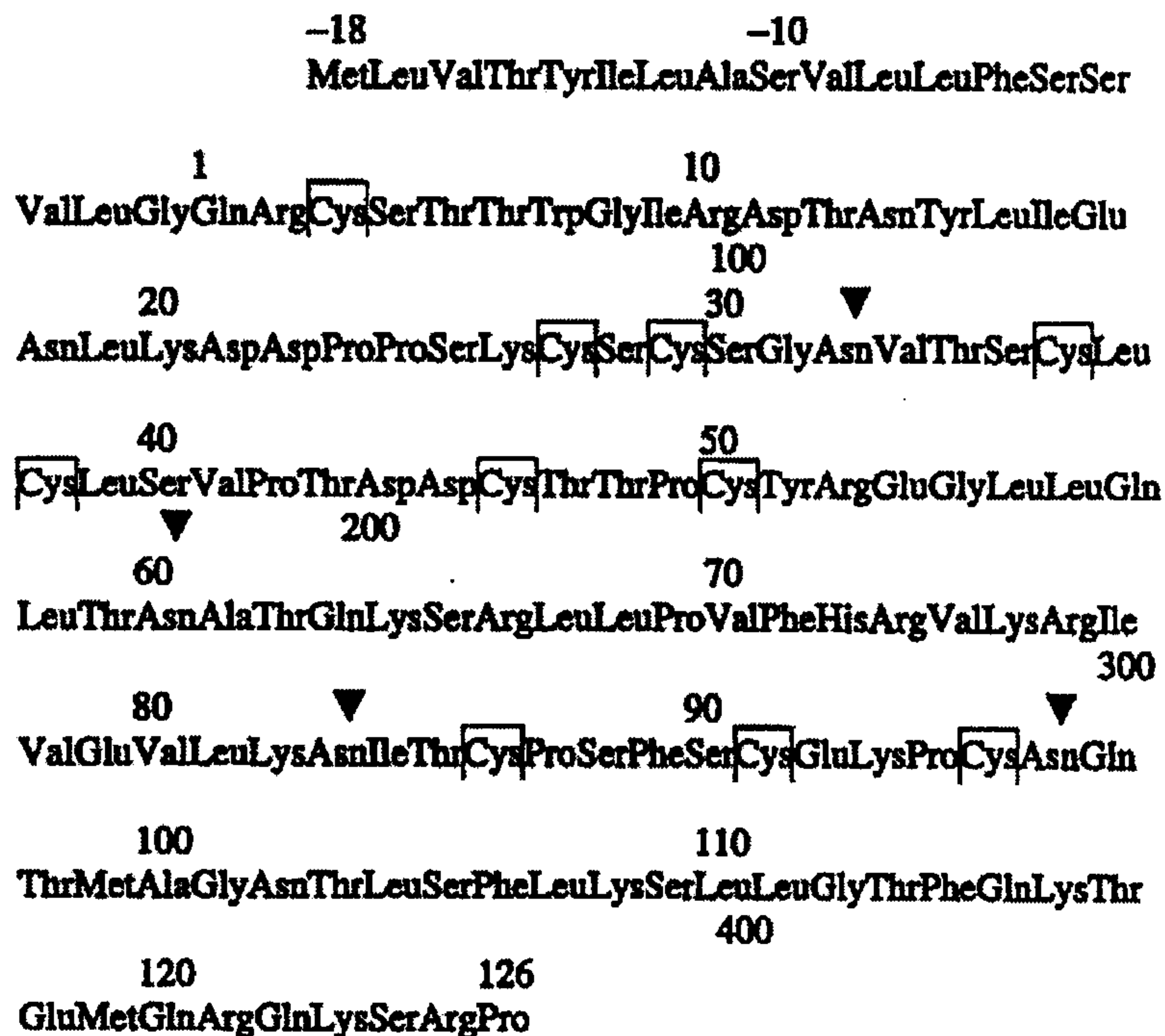
47. The nucleic acid of claim 46 wherein said cDNA has a nucleotide sequence encoding murine P40 which comprises:

5' CAGACTCCCGTCAACATGTTGGTGACATACATCCTTGCCTCTGTTTTGCTCTTCAGTTCT
 GTGCTGGGCCAGAGATGCAAGCACCACATGGGGCATCAGAGACACCAATTACCTTATTGAA
 100
 AATCTGAAGGATGATCCACCGTCAAAATGCAAGCTGCAAGCGGCAACGTGACCAGCTGCTTG
 TGTCTCTCCGTCCCAACTGATGATTGTACCACACCGTGTACAGGGAGGGACTGTTACAG
 200
 CTGACCAATGCCACACAGAAATCAAGACTCTTGCCTGTTTTCCATCGGGTGAAAAGGATA
 300
 GTTGAAGTCCTAAAGAACATCACGTGTCCGTCTTTTCTGCGGAAAAGCCATGGAACCAG
 ACCATGGCAGGCAACACACTGTCATTTCTGAAGAGTCTCCTGGGACGTTCCAGAAAGACA
 400
 GAGATGCAAAGGCAGAAAAGCCGACCATGAAGACAGATGCTATTTATTCTATTATTGAA
 TTTACAAAACCTCCCTCCTTAAGTGTACAGTGAAGAAATAAAGTAAAGCTATTCT 3'
 500

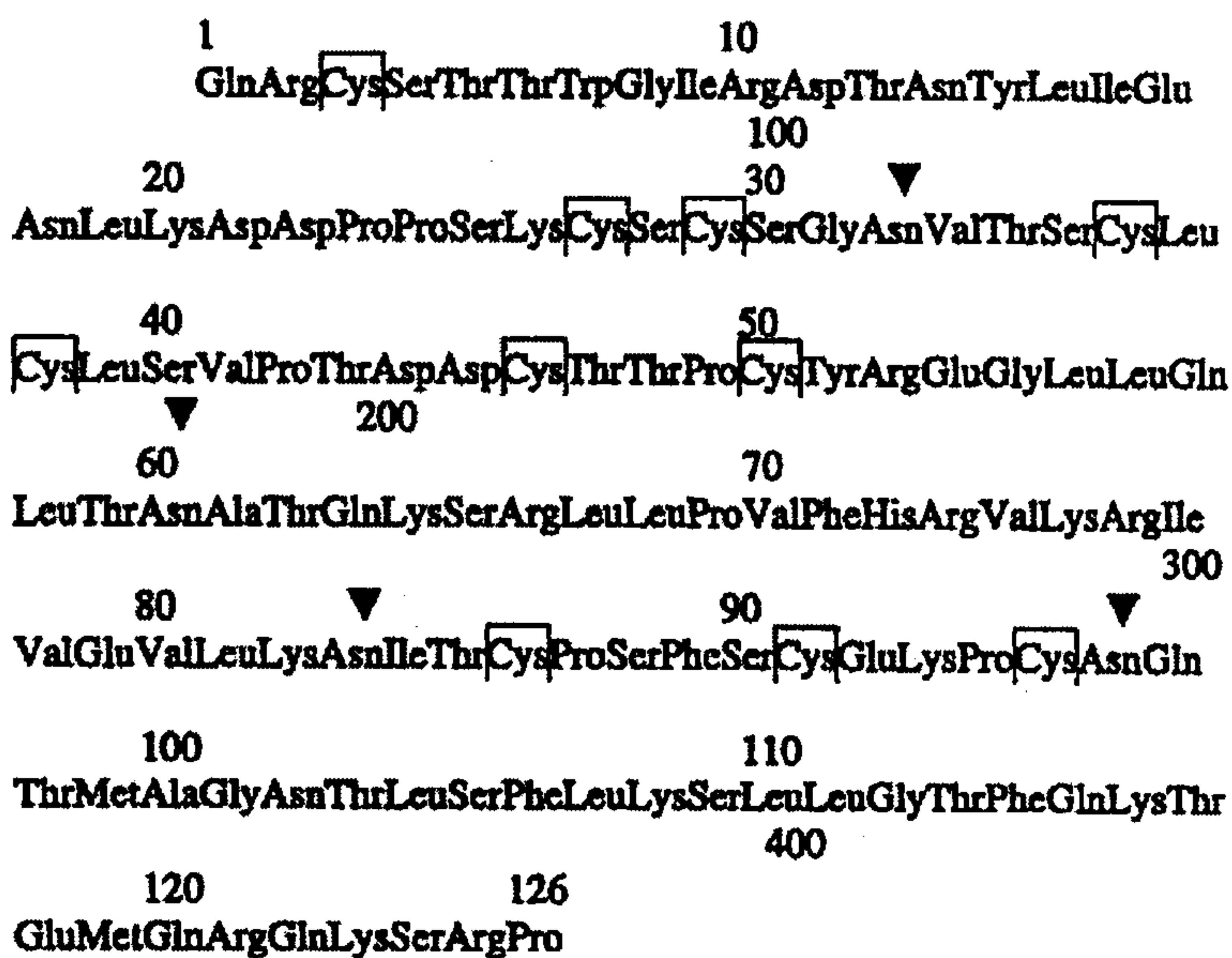
48. The nucleic acid of claim 46 wherein said cDNA has a nucleotide sequence encoding human P40 which comprises:

CCGCTGTCAAG ATG CTT CTG GCC ATG GTC CTT ACC TCT GCC CTG CTC CTG TGC TCC GTG GCA 62
 GGC CAG GGG TGT CCA ACC TTG GCG GGG ATC CTG GAC ATC AAC TTC CTC ATC AAC AAG ATG 122
 CAG GAA GAT CCA GCT TCC AAG TGC CAC TGC AGT GCT AAT GTG ACC AGT TGT CTC TGT TTG 182
 GGC ATT CCC TCT GAC AAC TGC ACC AGA CCA TGC TTC AGT GAG AGA CTG TCT CAG ATG ACC 242
 AAT ACC ACC ATG CAA ACA AGA TAC CCA CTG ATT TTC AGT CGG GTG AAA AAA TCA GTT GAA 302
 GTA CTA AAG AAC AAC AAG TGT CCA TAT TTT TCC TGT GAA CAG CCA TGC AAC CAA ACC ACG 362
 GCA GGC AAC GCG CTG ACA TTT CTG AAG AGT CTT CTG GAA ATT TTC CAG AAA GAA AAG ATG 422
 AGA GGG ATG AGA GGC AAG ATA TGAAGATGAAATATTATTTATCCTATTATTAAATTTAAAA 485

49. A nucleic acid having a nucleotide sequence encoding an amino acid sequence of murine P40 which comprises:



50. A nucleic acid having a nucleotide sequence encoding an amino acid sequence of murine P40 which comprises:



51. A nucleic acid having a nucleotide sequence encoding an amino acid sequence of human P40 which comprises:

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      -18
      MET LEU LEU ALA MET VAL LEU THR SER ALA LEU LEU LEU CYS SER VAL ALA
1      GLY GLN GLY CYS PRO THR LEU ALA GLY ILE LEU ASP ILE ASN PHE LEU ILE ASN LYS MET
20     GLN GLU ASP PRO ALA SER LYS CYS HIS CYS SER ALA ASN VAL THR SER CYS LEU CYS LEU
40     GLY ILE PRO SER ASP ASN CYS THR ARG PRO CYS PHE SER GLU ARG LEU SER GLN MET THR
60     ASN THR THR MET GLN THR ARG TYR PRO LEU ILE PHE SER ARG VAL LYS LYS SER VAL GLU
80     VAL LEU LYS ASN ASN LYS CYS PRO TYR PHE SER CYS GLU GLN PRO CYS ASN GLN THR THR
100    ALA GLY ASN ALA LEU THR PHE LEU LYS SER LEU LEU GLU ILE PHE GLN LYS GLU LYS MET
      126
ARG GLY MET ARG GLY LYS ILE

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52. A nucleic acid having a nucleotide sequence encoding an amino acid sequence of human P40 which comprises:

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1      GLN GLY CYS PRO THR LEU ALA GLY ILE LEU ASP ILE ASN PHE LEU ILE ASN LYS MET
20     GLN GLU ASP PRO ALA SER LYS CYS HIS CYS SER ALA ASN VAL THR SER CYS LEU CYS LEU
40     GLY ILE PRO SER ASP ASN CYS THR ARG PRO CYS PHE SER GLU ARG LEU SER GLN MET THR
60     ASN THR THR MET GLN THR ARG TYR PRO LEU ILE PHE SER ARG VAL LYS LYS SER VAL GLU
80     VAL LEU LYS ASN ASN LYS CYS PRO TYR PHE SER CYS GLU GLN PRO CYS ASN GLN THR THR
100    ALA GLY ASN ALA LEU THR PHE LEU LYS SER LEU LEU GLU ILE PHE GLN LYS GLU LYS MET
      126
ARG GLY MET ARG GLY LYS ILE

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53. A replicable expression vector comprising the nucleic acid of claim 45, wherein said nucleic acid is operably linked to a nucleotide sequence capable of effecting expression of said nucleic acid.
54. The vector of claim 53, wherein said vector is capable of replication in a eukaryote or prokaryote.
55. A microorganism or cell transformed by the nucleic acid of any one of claims 45-52.
56. A microorganism or cell transformed by the vector of claim 53.
57. Recombinant mammalian P40 or a derivative thereof.
58. Recombinant P40 of claim 57, wherein said P40 is biologically pure.
59. The P40 of claim 57, wherein mammalian is murine or human.
60. Recombinant human or murine P40 produced from the vector of claim 53.
61. A compartmentalized kit, for detection of P40, comprising: a first container containing an antibody to mammalian P40, an antigenic derivative thereof or an antigenic fragment thereof, and a second container adapted to contain a second antibody, wherein said second antibody is capable of detecting an antibody-antigen complex of said first antibody with said P40, said derivative or said fragment, and said second antibody is labeled with a reporter molecule and capable of giving a detectable signal.
62. The kit according to claim 61, wherein the reporter molecule is a radioisotope, an enzyme, a molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.
63. The kit according to claim 61, wherein the reporter molecule is an enzyme.

64. The kit according to claim 63, wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

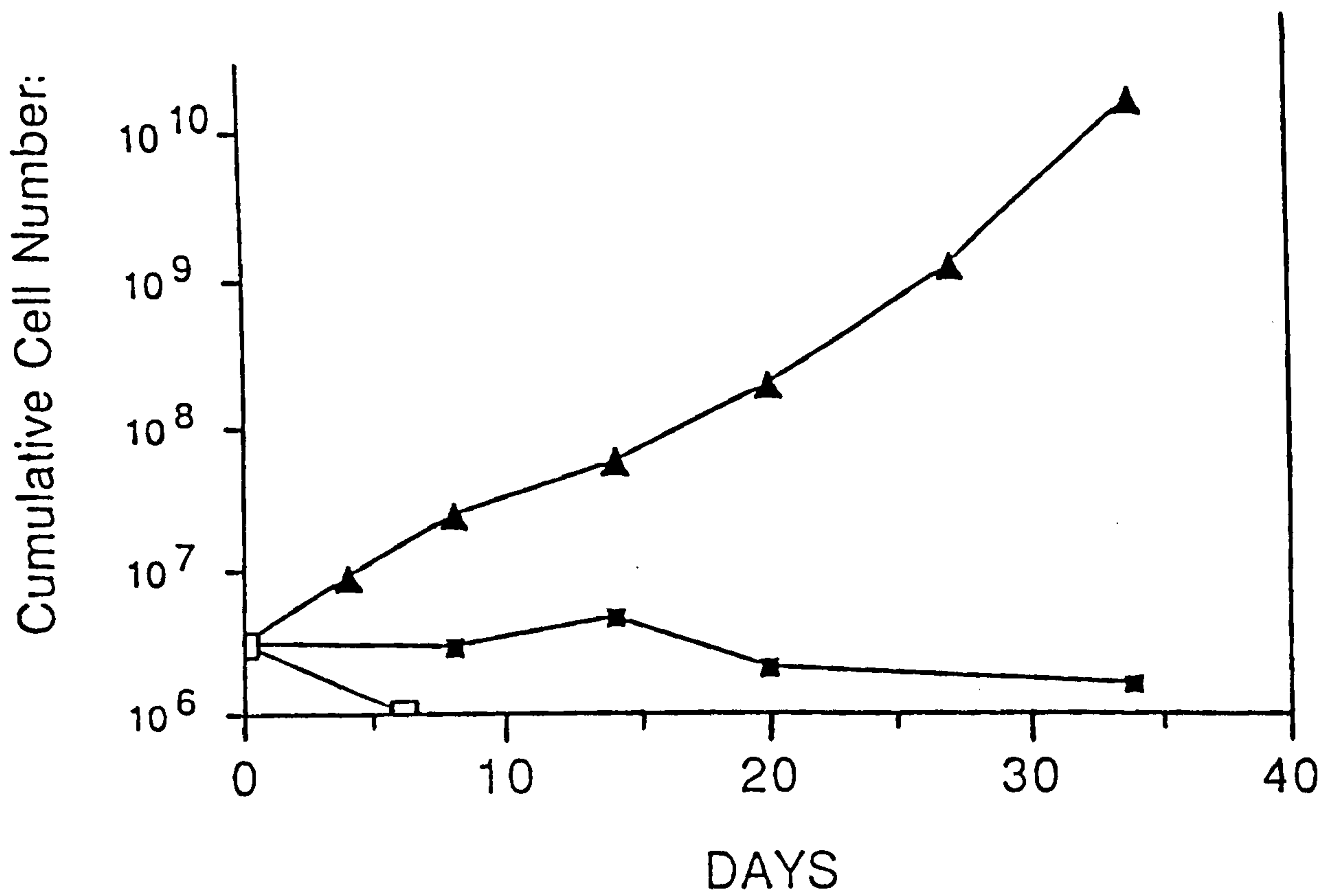


FIG. 1

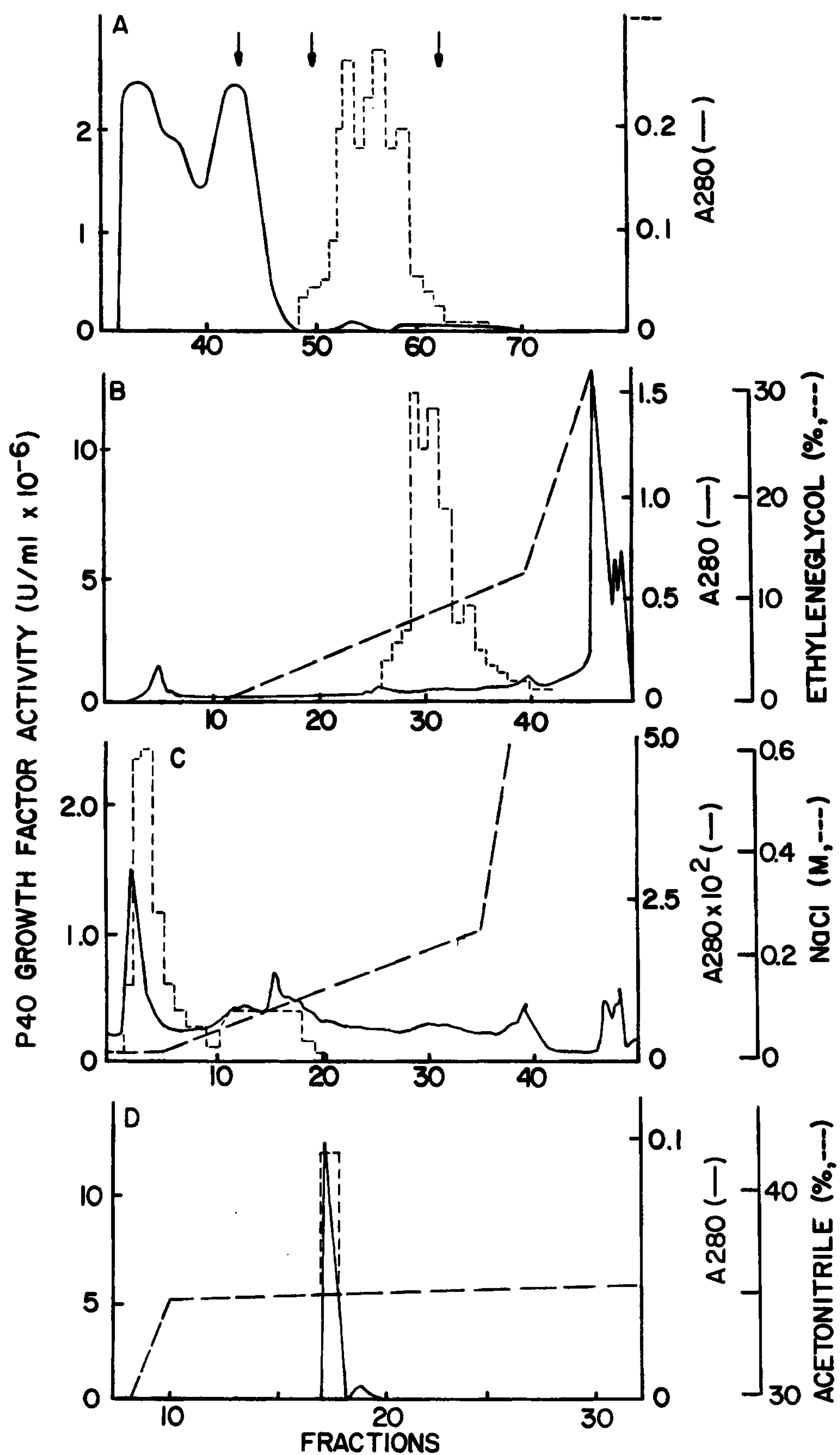


FIG. 2

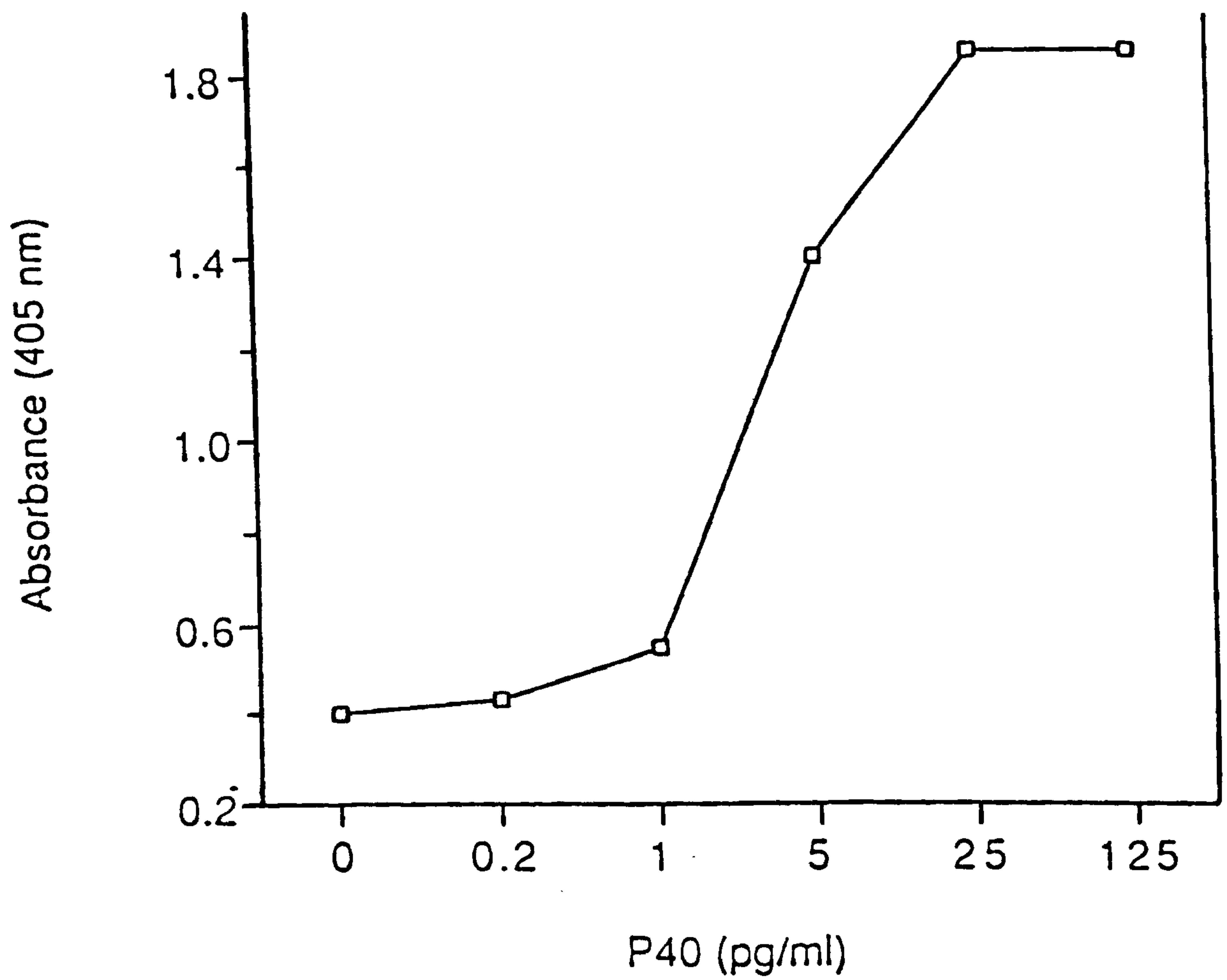


FIG. 3

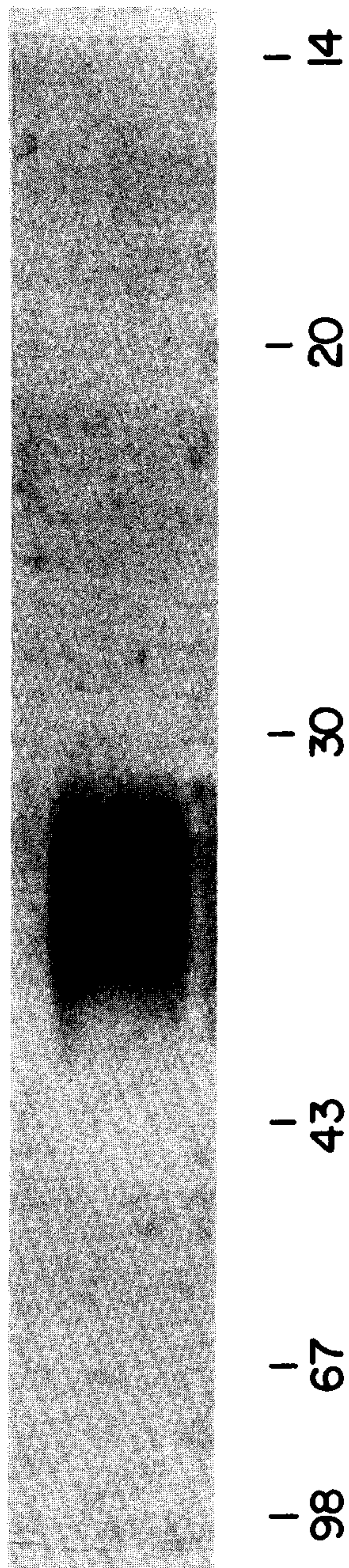
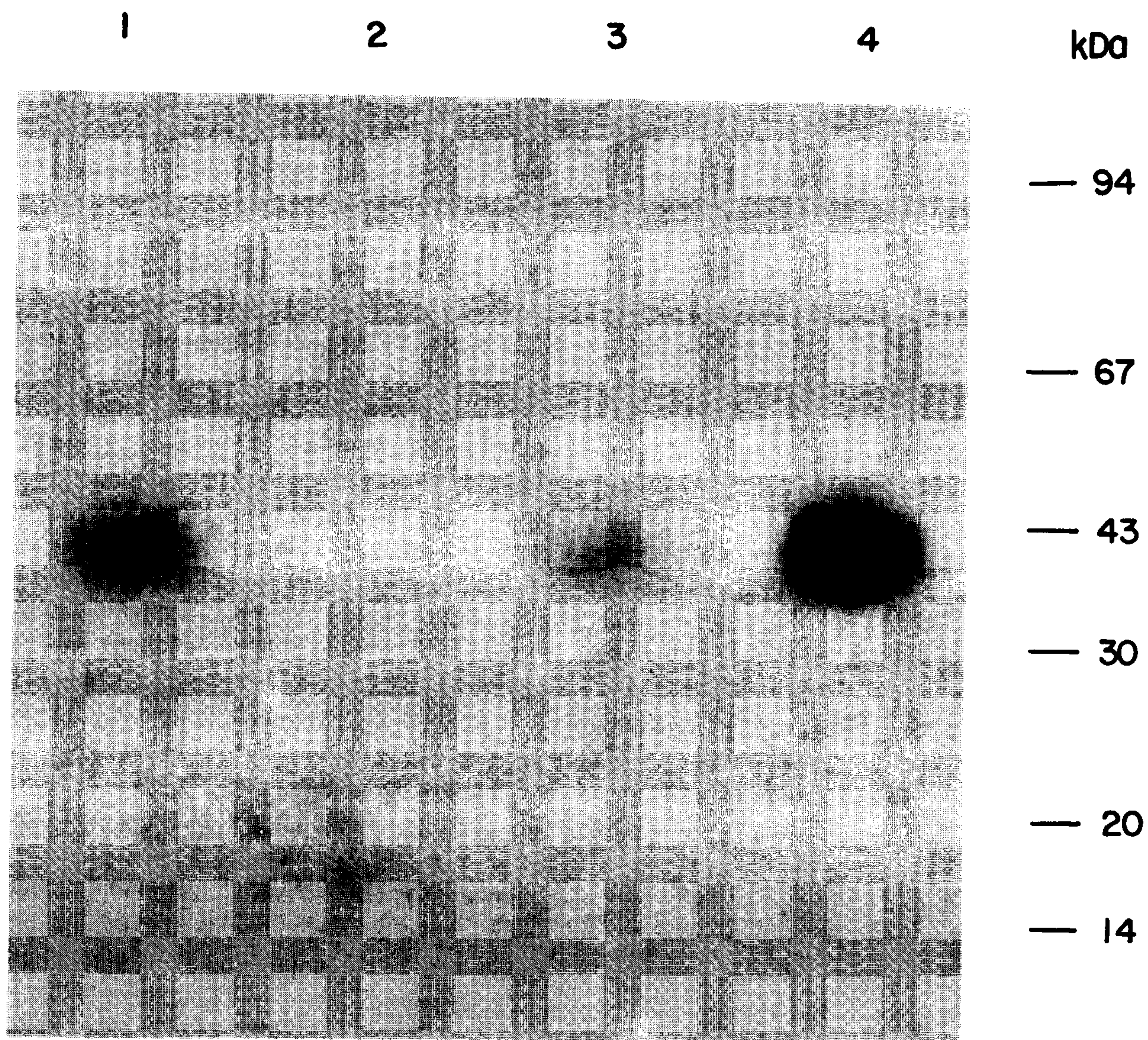


FIG. 4A

**FIG. 4B**

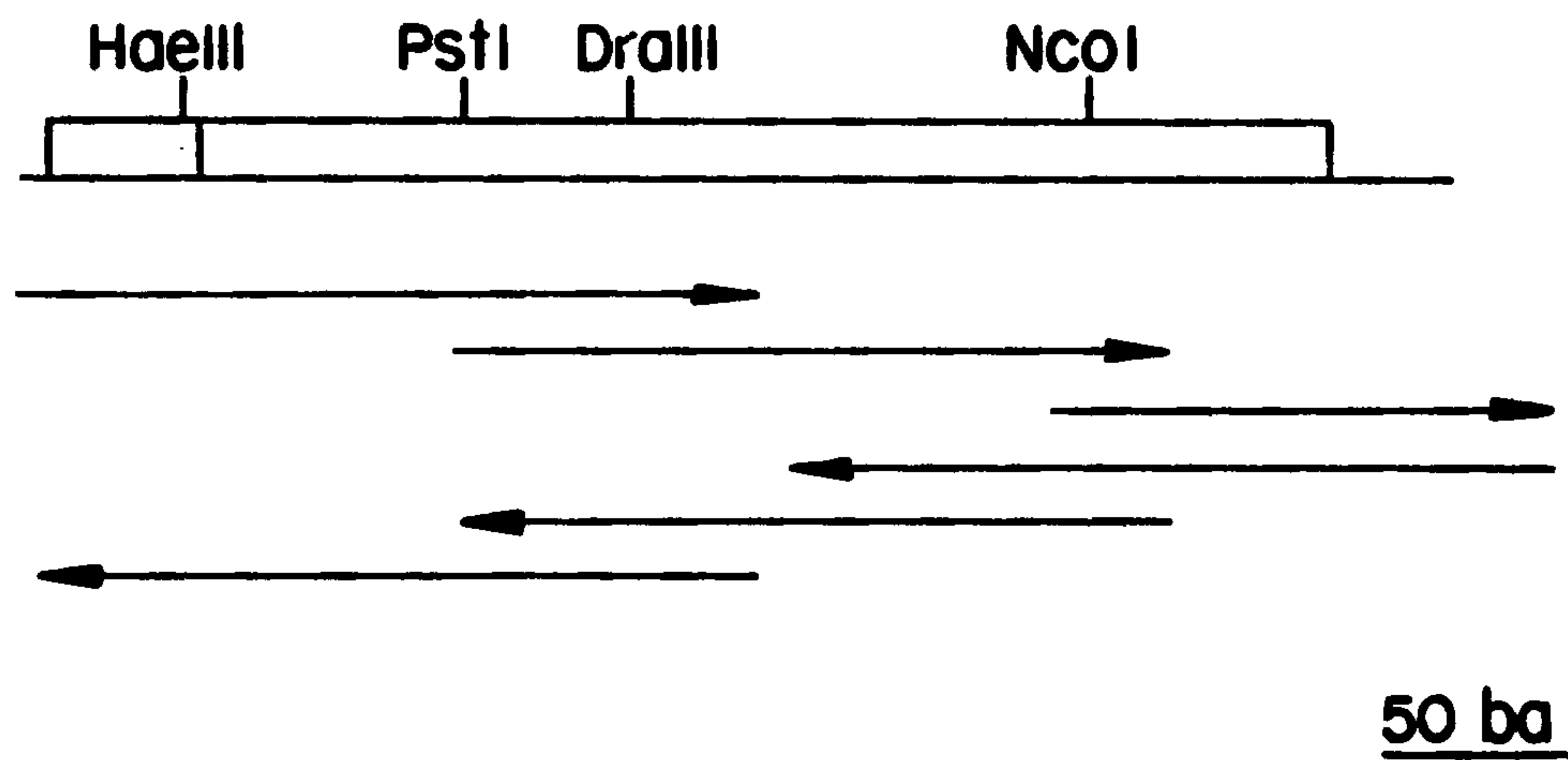


FIG. 5

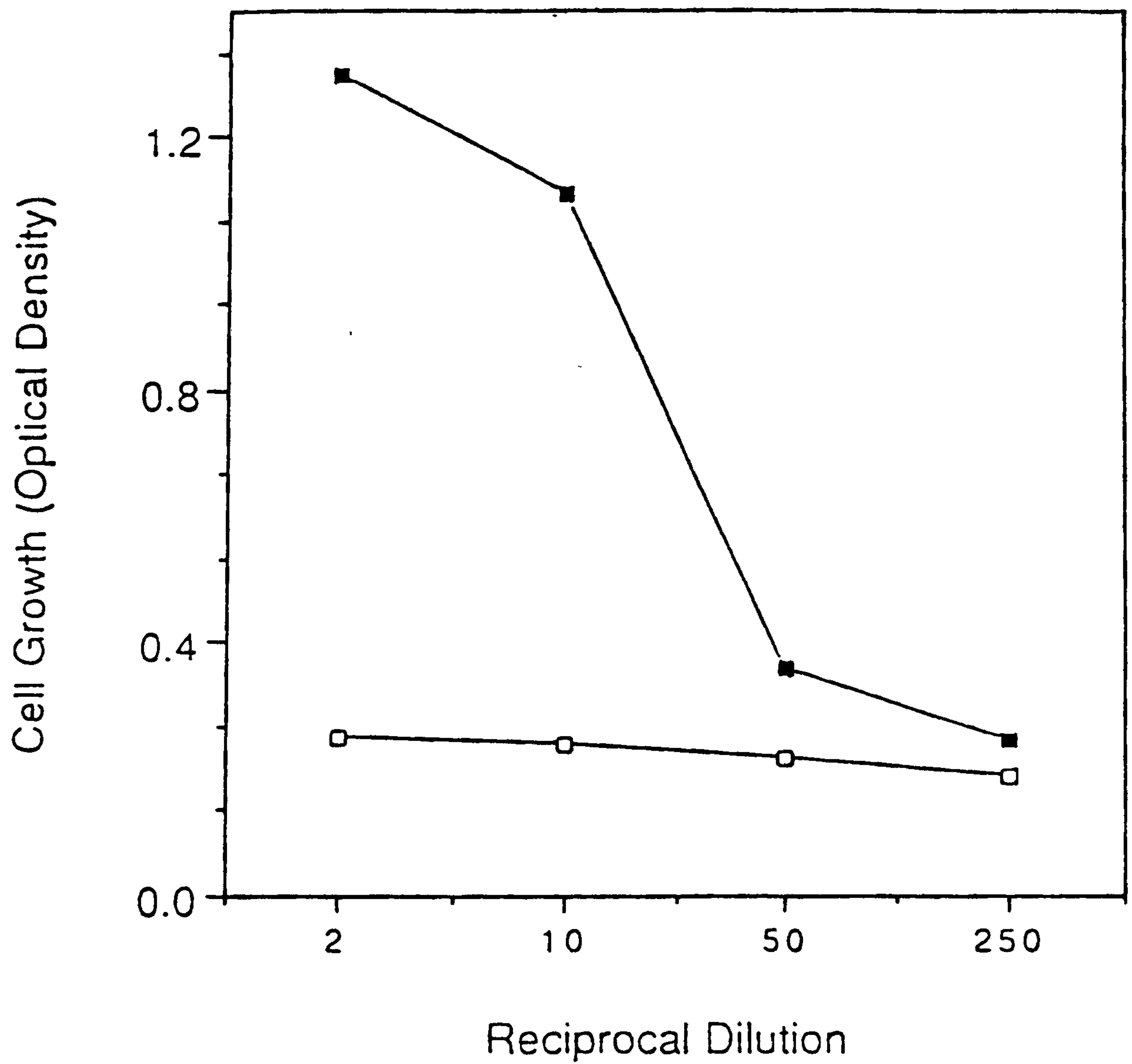


FIG. 6

CN2
qrqksrp

FIG. 7

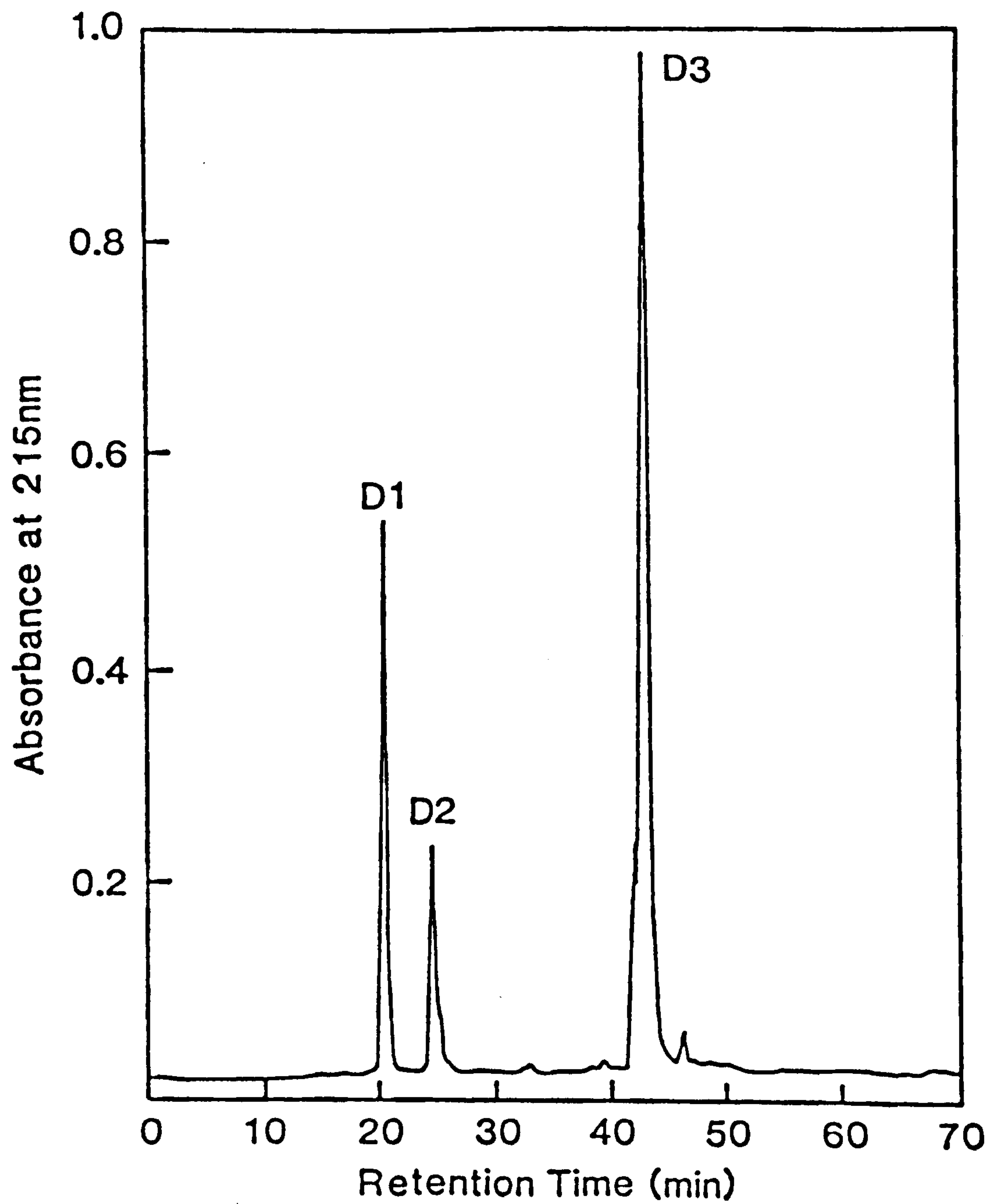


FIG. 8

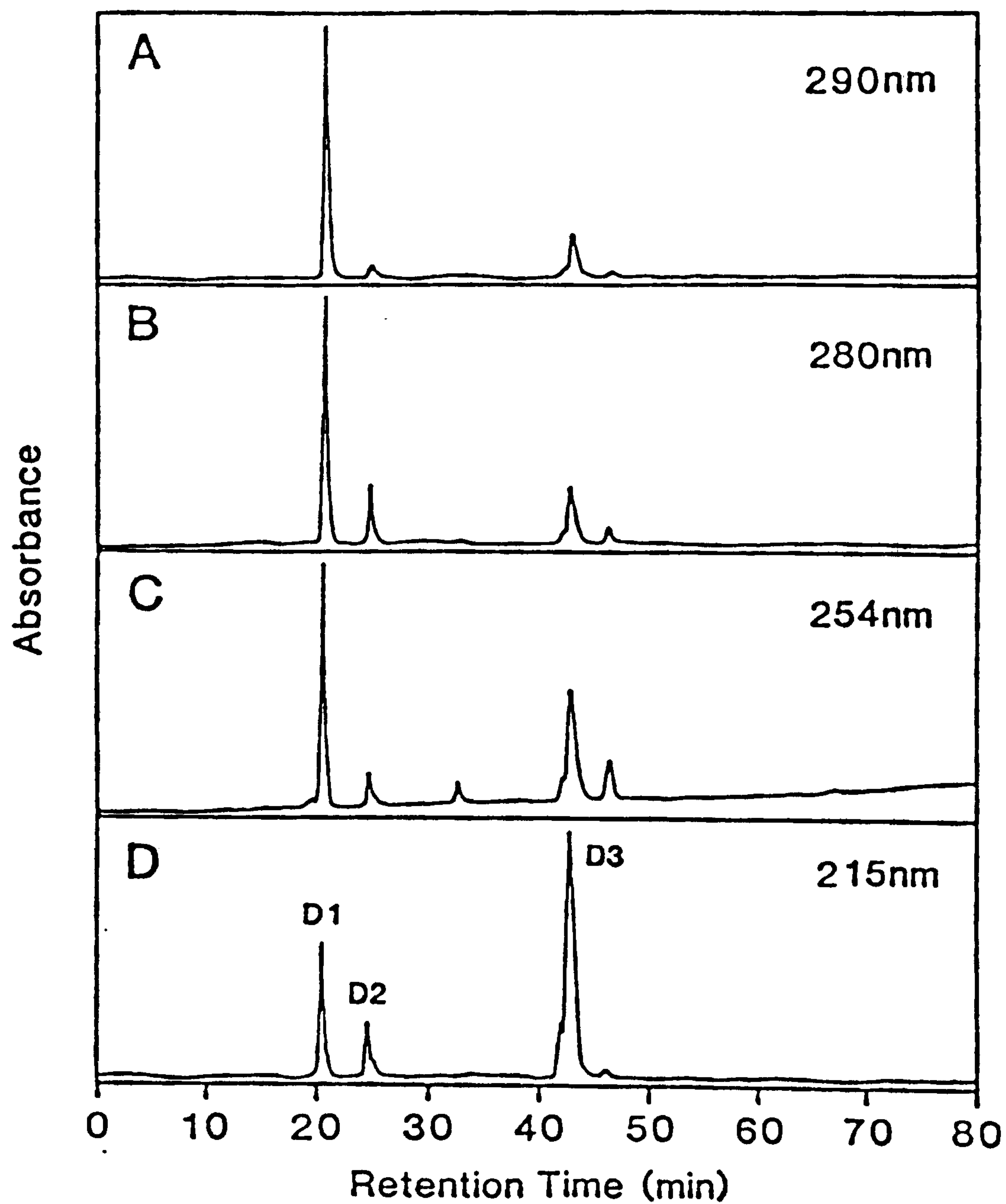


FIG. 9

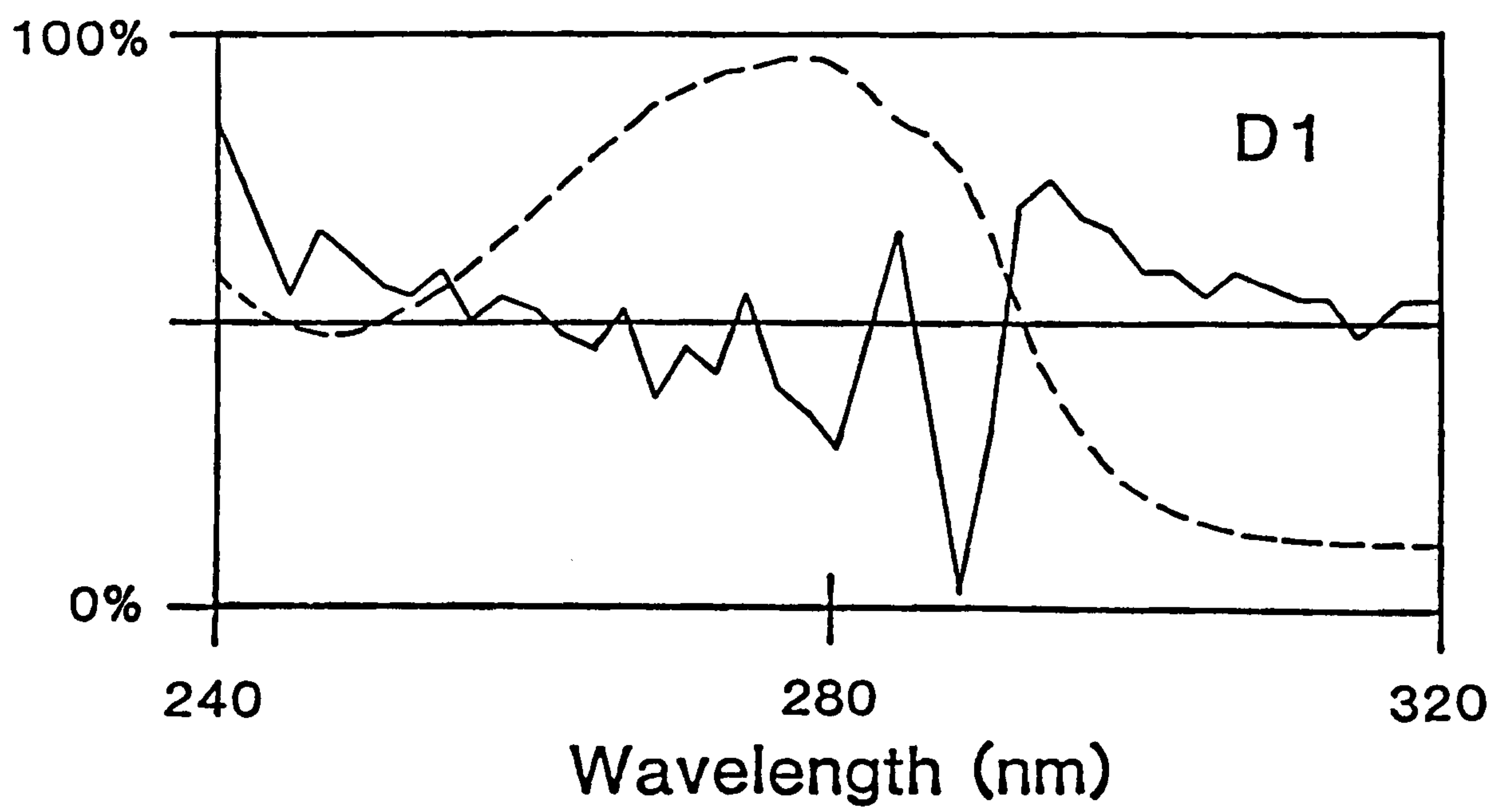


FIG. 10

FIG. IIA

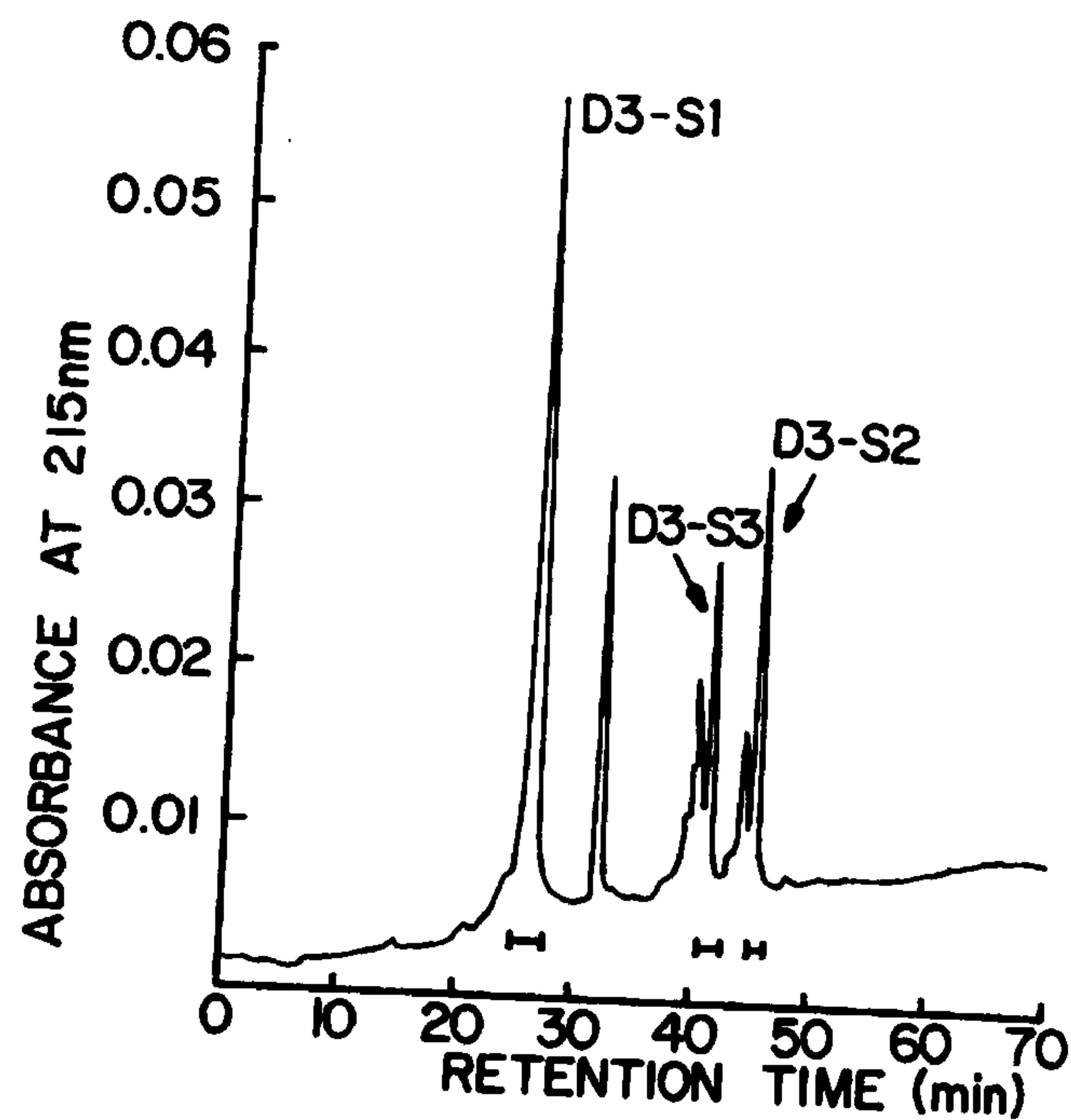


FIG. IIB

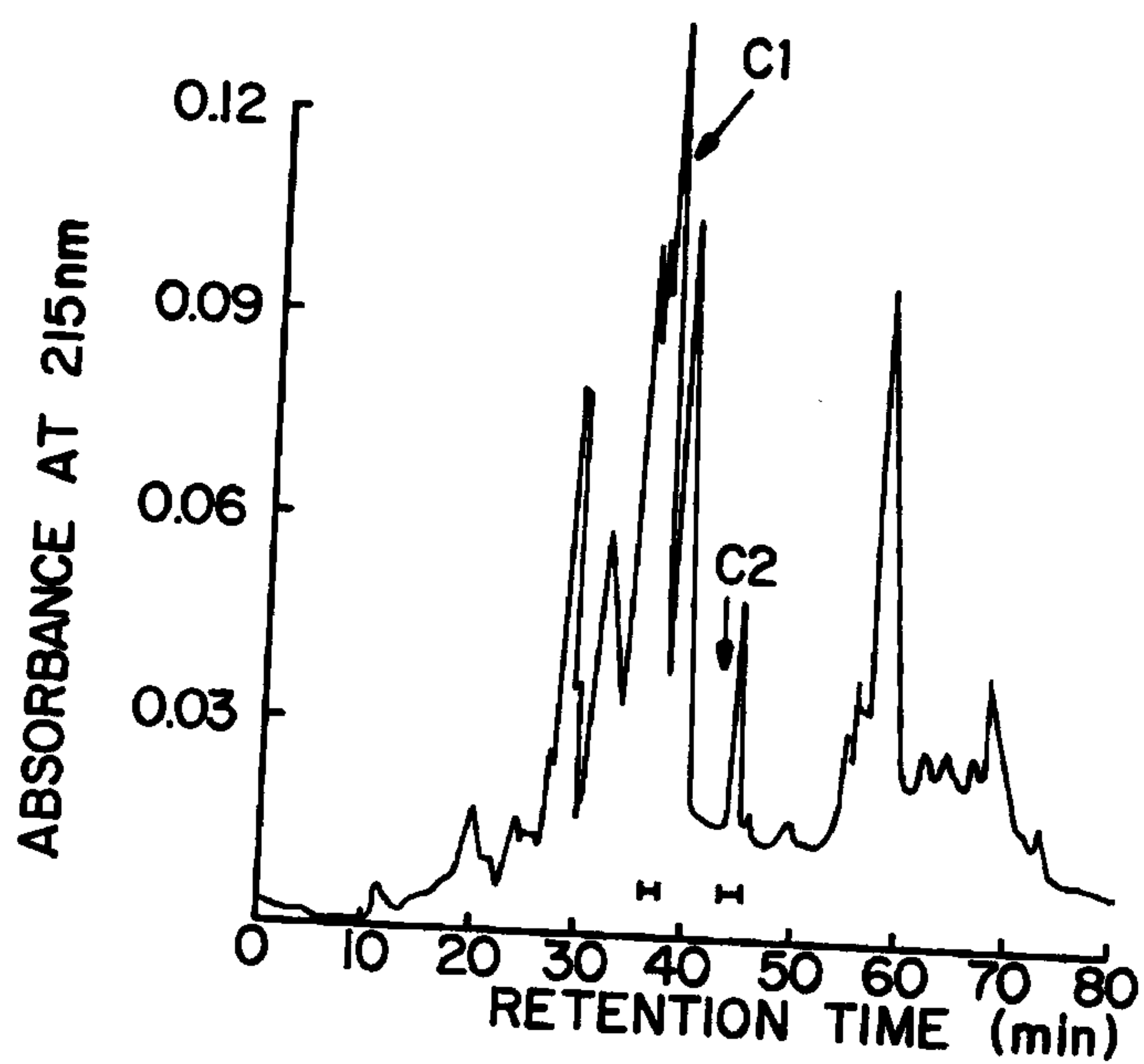
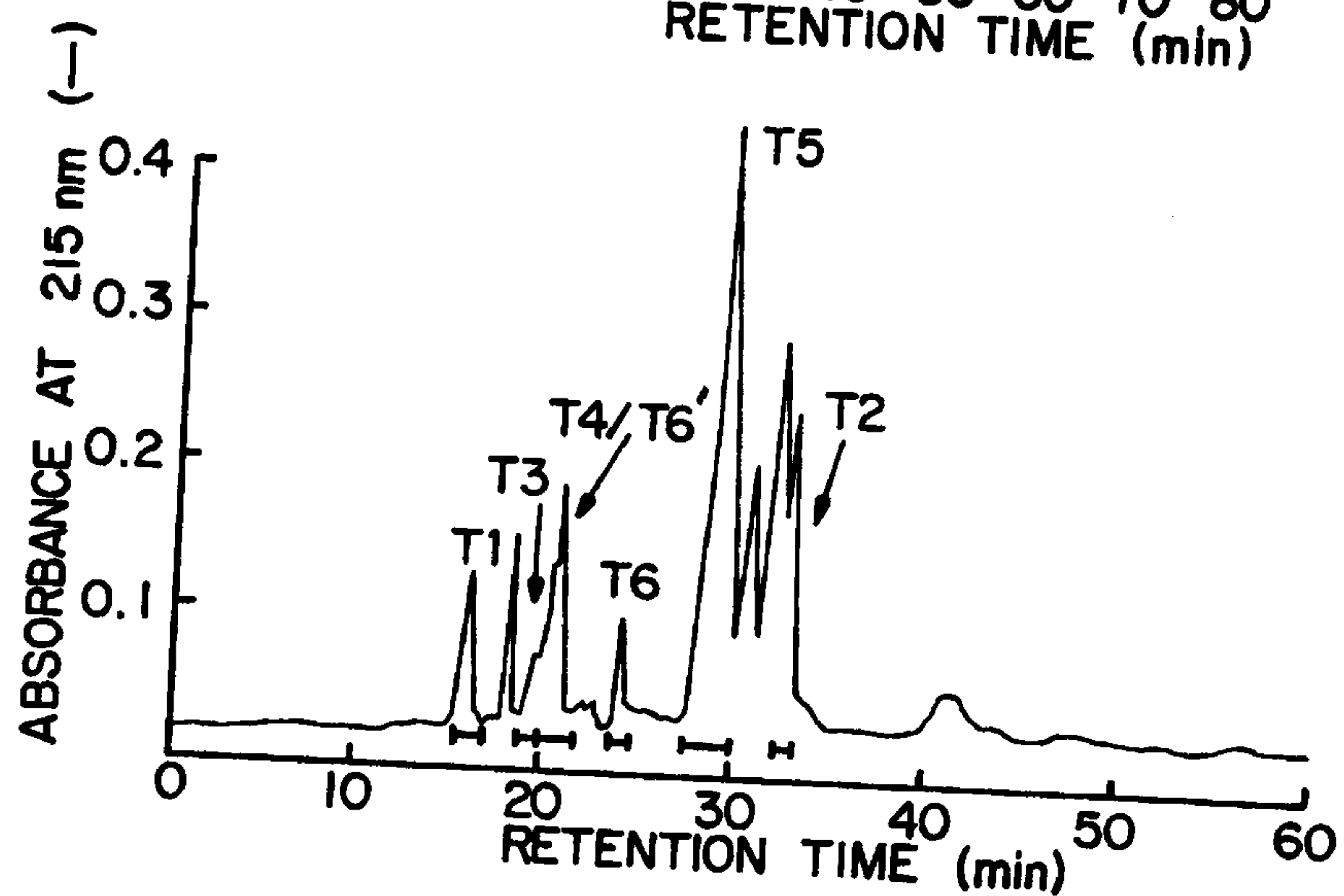


FIG. IIC



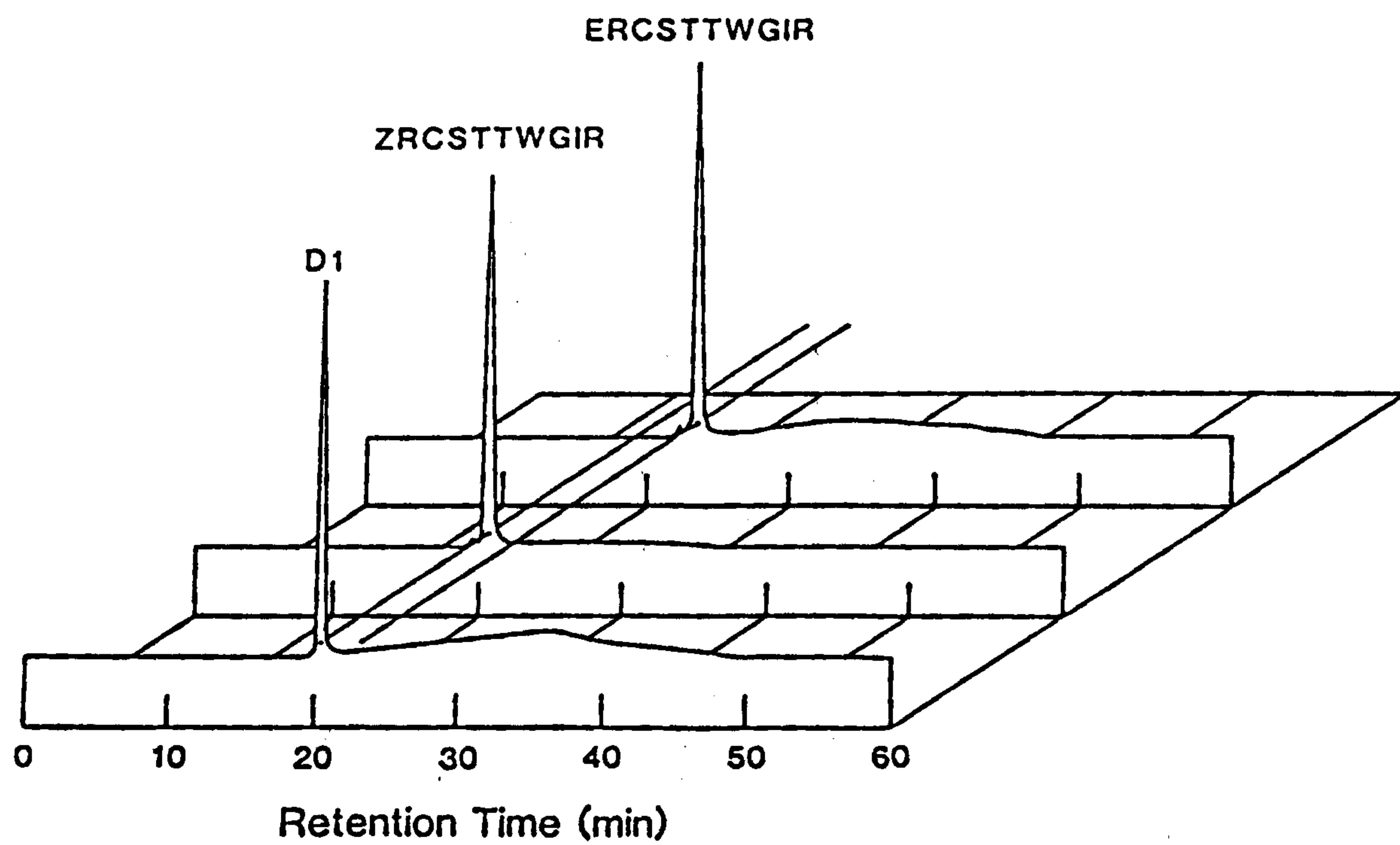


FIG. 12