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(54) **GENES TRANSPORTEURS**

(54) **TRANSPORTER GENES**

(57) On a réussi à isoler de nouveaux gènes majoritairement homologues des transporteurs de cations organiques OCT1 et OCT2, en triant une bibliothèque de gènes de fœtus par séquençage aléatoire. Des protéines codées par ces gènes servent de transporteurs de divers cations organiques en génétique appliquée.

(57) Novel genes significantly homologous with organic cation transporters OCT1 and OCT2 which have been successfully isolated by screening a fetal gene library by random sequencing. Proteins encoded by these genes serve as transporters of various organic cations in practice.

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ABSTRACT

Novel genes significantly homologous to organic cation transporters OCT1 and OCT2 have been successfully isolated by
5 screening a fetal gene library by random sequencing. Proteins encoded by these genes function as transporters of various organic cations.

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SPECIFICATION

TRANSPORTER GENES

5 Technical Field

The present invention relates to transporters, proteins involved in transport of substances from the outside to the inside of cells or vice versa.

10 Background Art

Recently, the involvement of various transporters localized on the plasmamembrane in the uptake system for nutrients and endogenous substances into cells and their transport mechanisms have been clarified (Tsuji, A. and Tamai, I., Pharm. Res., 13, 15 963-977, 1996). These transporters recognize the structures of substances to be transported to selectively transport specific substances. Transporters that recognize the relatively wide range of structures may possibly recognize foreign substances such as drugs by mistake, and actively take in them into cells. It 20 is believed that drugs permeate through the plasmamembrane fundamentally by simple diffusion depending on their physicochemical properties such as molecular size, hydrophobicity, and hydrogen-binding capacity. Particularly, in the case of ionic drugs, only molecules in the non-dissociated form can permeate 25 through the plasmamembrane according to the pH partition hypothesis. However, it has become evident that a number of drugs penetrate through the cell membrane by a specific mechanism other than simple diffusion, that is, an active transport mediated by transporters, in organs that require efficient exchange of 30 intracellular and extracellular substances, including small intestine, uriniferous tubule, placenta, epithelial cells of choroid plexus, hepatocytes, and blood-brain barrier (Tamai, I. and Tsuji, A., Pharmacia, 31, 493-497, 1995; Saito, H. and Inui, K., Igaku no Ayumi, 179, 393-397, 1996; Tamai, I., Yakubutsu Dotai

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(Pharmacokinetics), 11, 642-650, 1996). For example, it is known that although oral β -lactam antibiotics of the non-esterified type are amphoteric or negatively charged in physiological pHs and sparingly soluble in fat, they are readily absorbed through the intestine. The transport study using the isolated membrane-vesicles system demonstrated that an H^+ -driven peptide transporter localized on the brush-border is involved in the absorption process of these drugs (Tsuji, A. et al., J. Pharmacol. Exp. Ther., 241, 594-601, 1987). Although the specificity of a peptide transport system in terms of the peptide size is so strict as to recognize di- or tri-peptides but not tetrapeptides or larger peptides, it has a rather broad substrate specificity to recognize peptides comprising non-natural amino acids. The peptide transporter mediates transport of β -lactam antibiotics mistakenly due to its broad substrate specificity. This property has been unexpectedly utilized in the clinical field (Tsuji, A., American Chemical Society (eds. Taylor, M. D., Amidon, G. L.), Washington, D. C., 101-134, 1995). Furthermore, a possibility that a transporter is also involved in permeation of substances with a high hydrophobicity such as fatty acids through the plasmamembrane has been reported (Schaffer, J. and Lodish, H., Cell, 79, 427-436, 1994).

Since various transporters are supposed to be distributed in organs and cells based on the physiological roles of the organs and cells, their distribution and functions may be specific to organs. Therefore, transporters are expected to be used to impart an organ specificity to pharmacokinetics. In other words, an organ-specific drug delivery system (DDS) can be constructed utilizing transporters. If drug absorption solely relied on simple diffusion is improved by elevating its hydrophobicity, an effect of the drug obtained in the initial transport in the liver can be enhanced and the drug can non-specifically translocate into any organ. In addition, it would also be possible to increase the drug absorption independently of its fat-solubility by designing the drugutilizing the substrate specificity of transporters (Hayashi, K. et al., Drug Delivery System, 11, 205-213, 1996). For this purpose, it is necessary to identify

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various transporters at the molecular level and analyze their properties in detail. However, their molecular level identification are greatly behind studies on their membrane physiology because they are difficult to handle biochemically and
5 require complicated processes in their functional assays.

Recently, cDNAs of several transporters have been cloned by the expression cloning method using *Xenopus* oocytes, a foreign gene expression system, and structural homology among them has been revealed (Fei, Y.-J. et al., *Nature*, 368, 563-566, 1994).
10 For example, Koepsell et al. cloned an organic cation transporter, OCT1, which is assumed to be localized on a basement membrane, using the expression cloning method in 1994 (Grundemann, D. et al., *Nature*, 372, 549-552, 1994). Subsequently, OCT2 was identified by homology cloning based on the sequence of OCT1 (Okuda,
15 M. et al., *Biochem. Biophys. Res. Commun.*, 224, 500-507, 1996). OCT1 and OCT2 show homology as high as 67% to each other (Grundemann, D. et al., *J. Biol. Chem.*, 272, 10408-10413, 1997). Both of them are intensely expressed in the kidney, but differ in the organ distribution; OCT1 is also expressed in the liver, colon, and small
20 intestine, while OCT2 expression is specific to the kidney.

Only a few reports on identification of transporters at the molecular level, including the reports, are available, and there would be many unknown transporters that may be clinically useful.

25 Disclosure of the Invention

An object of this invention is to provide a family of novel transporter genes, proteins encoded by these genes, and their use.

The present inventors have screened a fetal gene library constructed using the subtractive method by random sequencing
30 based on a working hypothesis that fetal genes include those which are involved in various disorders including cancer and are specifically or intensely expressed in fetal tissues. The inventors discovered an unknown gene showing a significant homology with those for organic cation transporters, OCT1 and OCT2,
35 and attempted to isolate this gene, which was assumed to encode

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a novel transporter. Thus, the inventors succeeded in isolating the desired gene by screening a cDNA library derived from human fetus. Furthermore, the inventors studied the transporter activity of a protein encoded by the isolated human gene and found
5 that the protein, in fact, functioned as a transporter for various organic cations. The inventors also succeeded in isolating a mouse gene corresponding to the isolated human gene.

This invention relates to a family of novel transporter genes, proteins encoded by these genes, and their use, and more
10 specifically to:

- (1) a protein comprising an amino acid sequence set forth in SEQ ID NOs: 1, 3, 22, or 27, or a protein comprising said amino acid sequence in which one or more amino acid residues are substituted, deleted, or added, and having an activity to transport an organic
15 cation;
- (2) a protein encoded by a DNA hybridizing to a DNA comprising nucleotide sequence according to SEQ ID NOs: 2, 4, 23, or 28, and having an activity to transport an organic cation;
- (3) a DNA encoding the protein according to (1) or (2);
- 20 (4) a vector comprising the DNA according to (3);
- (5) a transformant expressibly carrying the DNA according to (3);
- (6) a method for producing the protein according to (1) or (2), the method comprising culturing the transformant according to (5);
- 25 (7) an antibody that binds to the protein according to (1) or (2); and
- (8) a DNA specifically hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NOs: 2, 4, 23, or 28, and consisting of at least 15 nucleotides.

30 Nucleotide sequences of cDNAs of novel human transporters isolated by the present inventors are shown in SEQ ID NO: 2 (designated as "human OCTN1") and SEQ ID NO: 4 (designated as "human OCTN2"), respectively. Amino acid sequences of proteins encoded by these cDNAs are shown in SEQ ID NO: 1 and SEQ ID NO: 3,

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respectively. Amino acid sequences of these two proteins included in the transporter proteins of this invention showed such a high overall homology as about 76%, and both of them retained the following consensus sequence which is conserved in various types of transporters including the glucose transporter: [Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala] (Maiden, M. C. et al., Nature, 325, 641-643, 1987). In fact, these proteins have an activity to transport various organic cations (see Examples 6 to 8).

The present inventors also isolated mouse genes corresponding to the above-described human OCTN1 and human OCTN2. Nucleotide sequences of the isolated cDNAs are shown in SEQ ID NO: 23 (designated as "mouse OCTN1") and SEQ ID NO: 28 (designated as "mouse OCTN2"), respectively. Amino acid sequences of proteins encoded by these cDNAs are shown in SEQ ID NOs: 22 and 27, respectively.

Transporter proteins of this invention also include those having the additional activity to transport substances other than organic cations as far as they retain the organic cation transport activity. Organic cations include, for example, TEA, carnitine, quinidine, and pyrilamine, but are not limited to them. They also include carcinostatic agents such as actinomycin D, etoposide, vinblastine, daunomycin, etc. Transporter proteins of this invention include those having the activity to transport organic cations not only from the outside to the inside of cells but also from the inside to the outside of cells.

Transporter proteins of this invention can be prepared as recombinant proteins using recombination techniques or natural proteins. Recombinant proteins can be prepared, for example, as described below, by culturing cells transformed with DNA encoding proteins of this invention. Natural proteins can be isolated from the kidney and cancer cell strains such as Hela S3, which highly express the proteins of this invention, by the method well known to those skilled in the art, for example, affinity chromatography

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using an antibody of this invention described below. The antibody may be either polyclonal or monoclonal. A polyclonal antibody can be prepared by purifying serum obtained from, for example, a small animal such as a rabbit immunized with proteins of this invention by known methods, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. A monoclonal antibody can be prepared by immunizing a small animal such as a mouse with the protein of this invention, excising the spleen from the mouse, grinding the tissue into cells, fusing them with mouse myeloma cells using a fusing agent such as polyethylene glycol, and selecting a clone that produces an antibody to proteins of this invention out of fused cells (hybridomas) thus produced. Then, hybridomas thus selected are transplanted into the abdominal cavity of a mouse, and the ascites is collected from the mouse. A monoclonal antibody thus obtained can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. When the antibody thus obtained is administered to human subjects, a humanized antibody or a human antibody is advantageously used to reduce the immunogenicity. An antibody can be humanized by, for example, the CDR grafting method comprising cloning an antibody gene from monoclonal antibody-producing cells and grafting the epitope portion thereof into an existing human antibody. A human antibody can be prepared by the usual method for preparing a monoclonal antibody except for immunizing a mouse whose immune system is replaced with the human's.

It is also possible for those skilled in the art to prepare proteins having functions equivalent to the transporter proteins of this invention (human OCTN1, human OCTN2, mouse OCTN1, and mouse OCTN2) by appropriately modifying amino acid residues of the proteins by, for example, substitution, using well known methods. Mutation of amino acids of the proteins can occur also spontaneously. Such mutant proteins which are obtained by altering the amino acid sequence of the transporter proteins of

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this invention by substitution, deletion, or addition of amino acid residues, and are functionally equivalent to those of the transporter proteins are also included in the proteins of this invention. Herein, "functionally equivalent" means that proteins have an activity to transport organic cations. Methods well known to those skilled in the art for altering amino acids include, for example, the site-specific mutagenesis system by PCR (GIBCO-BRL, Gaithersburg, Maryland), site-specific mutagenesis by oligonucleotide (Kramer, W. and Fritz, H. J. (1987) Methods in Enzymol., 154: 350-367), Kunkel's method (Methods Enzymol., 85, 2763-2766 (1988)), etc. The number of amino acids that can be substituted is usually 10 amino acid residues or less, preferably 6 or less, and more preferably 3 or less. The site of substitution, deletion, or addition of amino acid residues is not particularly limited as far as the activity of proteins of this invention is retained. It is possible to detect the transporter activity of proteins, for example, by the method described below in Example 6.

It is routine for those skilled in the art to obtain proteins functionally equivalent to the transporter proteins of this invention by isolating and using DNAs highly homologous to the DNA sequences encoding the transporter proteins of this invention (human OCTN1, human OCTN2, mouse OCTN1, and mouse OCTN2) or portions thereof using hybridization techniques (Sambrook, J. et al., Molecular Cloning 2nd ed., 9.47-9.58, Cold Spring Harbor Lab. press, 1989), etc. These proteins functionally equivalent to those of transporter proteins of this invention are also included in proteins of this invention. Here, "functionally equivalent" means that proteins have an activity to transport organic cations. DNAs that hybridize to the DNAs encoding the proteins of this invention can be isolated from other organisms, for example, rats, rabbits, cattle, etc. as well as humans and mice. Especially, tissues such as the kidney are suitable as sources of such DNAs. These DNAs isolated using hybridization techniques usually have a high homology with the above-described DNAs encoding the transporter proteins of this invention. "High homology" means at least 70% or more, preferably at least 80% or more, and more

preferably at least 90% or more of amino acid sequence identity. Sequence homology is determined according to algorithm described in a literature (Wilbur, W. J. and Lipman, D. J., Proc. Natl. Acad. Sci. USA (1983) 80, 726-730).

5 One example of hybridization conditions for isolating such DNAs is as follows. That is, after the pre-hybridization at 55°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 37°C to 55°C for 1
10 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS once at 37°C for 20 min. More preferable conditions are as follows. After the pre-hybridization at 60°C for 30 min or more in the "ExpressHyb
15 Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 60°C for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS twice at 50°C for 20 min.
20 Still more preferable conditions are as follows. After pre-hybridization at 68°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 68°C for 1 h or more. Then, the reaction product is successively washed
25 in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 0.1 x SSC and 0.1% SDS twice at 50°C for 20 min.

The present invention also relates to DNAs encoding the above-described transporter proteins of this invention. DNAs of this invention may be cDNA, genomic DNAs, and synthetic DNAs. The
30 DNAs of the present invention can be used for producing proteins of this invention as recombinant proteins. That is, it is possible to prepare proteins of this invention as recombinant proteins by inserting DNAs encoding proteins of this invention (e.g. DNAs comprising the nucleotide sequences set forth in SEQ ID NOs: 2,
35 4, 23, and 28) into an appropriate expression vector, culturing transformants obtained by transfecting suitable cells with the

vector, and purifying the proteins thus expressed. Cells to be used for producing recombinant proteins include, for example, mammalian cells such as COS cells, CHO cells, NIH3T3 cells, etc., insect cells such as Sf9 cells, yeast cells, *E. coli*, and so on.

5 Vectors used for the intracellular expression of recombinant proteins vary depending on host cells, including, for example, pcDNA3 (Invitrogen), pEF-BOS (Nucleic Acids Res., 1990, 18(7), p5322), etc. for mammalian cells, "BAC-to-BAC baculovirus expression system" (GIBCO BRL), etc. for insect cells, "Pichia

10 Expression Kit" (Invitrogen), etc. for yeast cells, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), etc. for *E. coli*. Host cells can be transformed with vectors, for example, by the calcium phosphate method, the DEAE-dextran method, the method using cationic liposome DOTAP (Boehringer Mannheim), the

15 electroporation method, the calcium chloride method, etc. Recombinant proteins can be purified from recombinants thus obtained using standard methods, for example, as described in "The Qiaexpressionist Handbook, Qiagen, Hilden, Germany."

The present invention also relates to DNAs consisting of at

20 least 15 nucleotides that specifically hybridize to the DNAs encoding proteins of this invention. Herein, "specifically hybridize" means that a DNA does not cross-hybridize to other DNAs encoding other proteins under usual hybridization conditions, preferably under the stringent hybridization conditions. Such

25 a DNA can be utilized as a probe for detecting and isolating DNA encoding the protein of this invention, and as a primer for amplifying the DNA.

The transporter proteins of this invention can be used to control internal absorption and dynamics of drugs. Based on the

30 results of detailed analysis of the substrate specificity of transporter proteins of this invention, drugs can be designed so as to be transported by these transporters and absorbability of the drugs mediated by these transporter proteins can be improved. Conventional modifications to enhance hydrophobicity are no

35 longer necessary for drugs so designed, which enables speedily and efficiently developing water-soluble drugs that are easy to

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handle. The drugs thus developed is thought to be absorbed principally depending on the internal distribution pattern of transporter proteins of this invention, and an organ-specific delivery of the drugs thus becomes possible. Especially, if the
5 transporter proteins of this invention are distributed in the target organ of a drug, an ideal drug delivery system (DDS) can be developed. If a drug is to be absorbed mediated by not the transporter proteins of this invention but other transporters, the drug can be designed so as to be specific to other transporter
10 proteins by designing it considering the substrate specificity of the transporter proteins of this invention. Since the transporter proteins of this invention are present in the kidney, it is possible to reduce the nephrotoxicity produced by a drug by designing the drug so that it can be readily excreted by the
15 transporter proteins of this invention.

Another possible application of this invention is to develop a drug targeting the transporter proteins of this invention. The transporters play important roles in the absorption mechanism of nutrients and drugs, or the excretion mechanism of drugs and
20 internal metabolites. Thus, damage or abnormal elevation of the transporter's functions may cause some disorders. It is considered to be efficacious against such disorders to administer a drug containing a compound that inhibits or enhances functions of the transporter proteins of this invention, or regulates the
25 expression level of the transporter gene of this invention and the amount of the transporter proteins. The DNAs of this invention can be used in gene therapy for disorders caused by abnormalities in the activity and expression of the proteins of this invention. In this case, the DNA of this invention are inserted to an
30 adenovirus vector (e.g. pAdexLcw), a retrovirus vector (e.g. pZIPneo), etc., and administered into the body by either *ex vivo* method or *in vivo* method. Gene therapy can also be performed by administering a synthetic antisense DNA to the body either directly or after inserted into the above-described vector.

35 Especially, since "OCTN2" included in the transporter proteins of this invention efficiently transports carnitine,

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chemotherapy with compounds to control the activity of "OCTN2" or gene therapy using the "OCTN2" gene is considered to be efficacious against various pathological conditions such as fatty liver, myocardiopathy, myopathy, etc. caused by hypocarnitinemia.

5 The transporter proteins of this invention are expressed in a variety of cancer cell strains, which suggests that the proteins may transport drugs into tumor cells. If this is the case, it is possible to develop carcinostatics that will be readily absorbed mediated by the transporter proteins of this invention.

10 On the contrary, mechanisms to transport and excrete substances by the transporter proteins of this invention may function to excrete carcinostatics in tumor cells so that the cells acquire resistance to drugs. If the transporter proteins of this invention are involved in a mechanism of tumor cells to acquire

15 drug resistance, a carcinostatic effect can be enhanced by a combined use of inhibitors of the transporter proteins of this invention with carcinostatics.

Brief Description of the Drawings

20 Fig. 1 represents hydrophobicity plots of human OCTN1 and human OCTN2 according to Kyte & Doolittle's calculating formula with a window of nine amino acid residues. Numerals on the plots indicate putative transmembrane regions.

 Fig. 2 represents electrophoretic patterns showing the

25 results of Northern blot analysis of human OCTN1.

 Fig. 3 compares the amino acid sequence of human OCTN1 with that of human OCTN2. Amino acid residues conserved in both transporters are shaded. Sequences coinciding with the consensus sequences of sugar transporter and the ATP/GTP binding site are

30 indicated by "+" and "*"," respectively.

 Fig. 4 represents electrophoretic patterns showing the results of Northern blot analysis of human OCTN2.

 Fig. 5 is a graph showing the TEA-absorbing activity of human OCTN1. Clear circles represent untreated cells, and solid circles

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represent human OCTN1-transfected cells.

Fig. 6 is a graph showing effects of the cold TEA added in the experimental system in Fig. 5. In this graph, solid circles represent human OCTN1-transfected cells, and clear circles represent cells containing the vector with no insert. Clear triangles indicate the net uptake induced by human OCTN1 obtained by subtracting the clear circle values from the corresponding solid circle values.

Fig. 7 is a graph showing TEA concentration-dependency of the TEA-absorbing activity of human OCTN1.

Fig. 8 is a bar graph showing the activity of the human OCTN1-transfected cells to absorb substances other than TEA.

Fig. 9 is a bar graph showing the results of transport experiments using *Xenopus* oocytes. Bars indicated with "OCTN1" and "Water" represent the uptake activity of the human OCTN1-injected cRNA oocytes and that of the water-injected oocytes (containing no cRNA), respectively. Uptakes of TEA, carnitine, mepyramine, quinidine, and actinomycin D were observed in human OCTN1 cRNA-injected oocytes, whereas water-injected oocytes (containing no cRNA) exhibited almost no uptake activity.

Fig. 10 is a bar graph showing the results of transport experiments for carcinostatics in *Xenopus* oocytes. Bars indicated with "OCTN1" and "Water" represent the uptake activity of the human OCTN1 cRNA-injected oocytes and that of the water-injected oocytes (containing no cRNA), respectively. Uptakes of actinomycin D, etoposide, vinblastine, and daunomycin were observed in the human OCTN1 cRNA-injected oocytes.

Fig. 11 is a bar graph showing the results of transport experiments with human OCTN1 and human OCTN2 in HEK293 cells. Human OCTN1 has the efficient transport activity for TEA and human OCTN2 for carnitine.

Fig. 12 is a graph showing the results of Na⁺-dependency of the carnitine transport activity of human OCTN2. Human OCTN2 exhibits a time-dependent carnitine transport activity (clear circle) in the presence of Na⁺, while no such activity in the absence

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of Na⁺ (solid circle), indicating that the carnitine transport activity of human OCTN2 depends on the presence of Na⁺.

Fig. 13 shows the expression of mouse OCTN1 and mouse OCTN2 genes detected by RT-PCR amplification in each tissue. G3PDH serves as a control, indicating that the amount of cDNA in each tissue is uniform.

Best Mode for Carrying out the Invention

The present invention is described below in more detail with reference to examples, but is not construed being limited thereto.

Example 1 Construction of a subtraction library

A subtraction library was constructed using the PCR-SelectTM cDNA Subtraction Kit (CLONTECH) principally according to the method of Luda Diatchenko (Diatchenko, L. et al., Proc. Natl. Acad. Sci. USA, 93, 6025-6030, 1996).

First, double-stranded cDNAs were synthesized from poly(A)⁺ RNAs derived from human fetal liver and adult liver by the standard method using MMLV reverse transcriptase. These cDNAs were blunt-ended with T4 DNA polymerase and cleaved with RsaI. A part of the cDNAs derived from fetal liver (tester) was divided in two portions, and they were separately ligated to two different adapters, adapter 1 and adapter 2, respectively (Table 1). A 120-fold excess of cDNA derived from adult liver (driver) was added to each of the above-described tester samples. The mixture was heat-denatured and subjected to the primary hybridization at 68°C for 8 h. After these two reaction mixtures from the primary hybridization were mixed together without heat-denaturation, an excessive amount of the heat-denatured driver was further added thereto, and the mixture was subjected to the secondary hybridization at 68°C for about 16 h. The resulting reaction solution was diluted with a dilution buffer and incubated at 75°C for 7 min. After the shorter strands of adapters were removed, the reaction solution was used as a template for PCR. PCR using primers 1 (5'-CTAATACGACTCACTATAGGGC-3', SEQ ID NO: 5) and 2 (5'-TGTAGCGTGAAGACGACAGAA-3', SEQ ID NO: 6) corresponding to the

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adapters selectively amplified only cDNAs having different adapters at their both ends (subtracted cDNAs) (suppression PCR). PCR was carried out using a portion of the resulting cDNA as a template, and nested PCR primers 1 (5'-TCGAGCGGCCGCCCCGGGCAGGT-3', SEQ ID NO: 7) and 2 (5'-AGGGCGTGGTGCGGAGGGCGGT-3', SEQ ID NO: 8), which are further inwardly located from the PCR primers 1 and 2, to obtain products with further elevated selectivity. PCR products thus obtained were purified using the QIAquick PCR Purification kit (QIAGEN), and cloned into the pT7Blue-T vector (Novagen) by the TA cloning method to construct a subtraction library.

Table 1

Adapter 1	5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCCGGGCAGGT-3' 3'-GGCCCGTCCA-5'
Adapter 2	5'-TGTAGCGTGAAGACGACAGAAAGGGCGTGGTGCGGAGGGCGGT-3' 3'-GCCTCCCGCCA-5'

Example 2 cDNA cloning

To analyze fetal genes, the subtraction library derived from the fetal liver was screened by random sequencing. Homology search (Blastx) of Expressed Sequence Tags (ESTs) thus obtained found a clone, OCTN1 (fls 631) (292 bp) encoding amino acid sequence having significant homology with the known organic cation transporters, OCT1 (Grundemann, D. et al., Nature, 372, 549-552, 1994) and OCT2 (Okuda, M. et al., Biochem. Biophys. Res. Commun., 224, 500-507, 1996). Since the sequence of this clone was novel and assumed to be a fragment derived from a new transporter gene, cDNA comprising the whole open reading frame (ORF) of this gene was cloned.

The human fetal liver 5'-stretch cDNA library (CLONTECH) was screened using the original OCTN1 clone obtained from the subtraction library derived from fetal liver as a probe. An insert of the original OCTN1 clone was amplified by PCR using M13 P4-22 and M13 P5-22, and labeled with [α -³²P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to

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serve as a probe. Hybridization was carried out at 68°C in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50°C in 0.1 x SSC and 0.1% SDS. Screening about 5 x 10⁵ phage clones finally isolated seven positive clones. cDNA inserts of these clones were amplified by PCR using vector primers designed based on a sequence of the λ gt10 vector (GT10 S1 5'-CTTTTGAGCAAGTTCAGCCT-3', SEQ ID NO: 9, and GT10 A1 5'-AGAGGTGGCTTATGAGTATTTCTT-3', SEQ ID NO: 10), or primers designed based on the decoded cDNA sequences. The PCR products thus obtained were directly sequenced to determine the nucleotide sequences. Some regions that were difficult to be amplified were subjected to PCR using 7-deaza dGTP as a substrate base (McConlogue, L. et al., Nucleic Acids Res., 16, 9869, 1988).

Sequencing of cDNA inserts of these clones revealed that the human OCTN1 gene contains an ORF encoding a protein consisting of 551 amino acid residues (putative molecular weight of about 62,000). Data base search using this whole amino acid sequence confirmed that it has a significant overall homology (about 34%) with OCT1 and OCT2. Hydrophobicity profile of this sequence obtained by Kyte & Doolittle's calculating formula (Kyte, J. and Doolittle, R. F., J. Mol. Biol., 157, 105-132, 1982) very closely resembled those of OCT1 and OCT2, indicating that the sequence has eleven to twelve putative transmembrane hydrophobic regions (Fig. 1). This sequence contained one consensus sequence of sugar transporter, ([Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala]), (160 to 175). This consensus sequence is present in the glucose transporters GLUT1 to GLUT7 in mammalian cells, and also present in various types of transporters other than glucose transporters (Maiden, M. C. et al., Nature, 325, 641-643, 1987). Furthermore, putative N-linked glycosylation sequences (N-X-[ST]) were found in the amino acid sequence of human OCTN1 at four sites (57 to 59, 64 to 66, 91 to 93, and 304 to 306), and also five putative protein kinase C phosphorylation sites ([ST]-X-[RK]) (164 to 166, 225 to

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227, 280 to 282, 286 to 288, and 530 to 532). In addition, the consensus sequence ([Ala, Gly]-Xaa(4)-Gly-Lys-[Ser, Thr]) of the ATP/GTP binding site is also found. This consensus sequence of the ATP/GTP binding site is also present in the ATP binding protein or GTP binding protein, such as kinases and ras family proteins, and that ATP or GTP binds to this site (Walker, J. E. et al., EMBO J., 1, 945-951, 1982). This sequence is present in the so-called ATP Binding Cassette (ABC) type transporter, and involved in the substance transport using the energy generated by hydrolysis of ATP (Higgins, C. F. et al., J. Bioenerg. Biomembr., 22, 571-592, 1990; Urbatsch, I. L. et al., J. Biol. Chem., 270, 26956-26961, 1995). Presence of this consensus sequence indicates that OCTN1 protein may be an ATP or GTP-dependent transporter.

Nucleotide sequencing was performed by the cycle-sequencing method with a plasmid DNA prepared by the alkaline-SDS method or a PCR product obtained by colony PCR, etc. as a template using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit With AmplyTaq DNA Polymerase, FS, followed by decoding with the ABI 377 DNA Sequencer (Perkin Elmer). Colony PCR was carried out by directly suspending a colony of a recombinant in a PCR reaction solution containing vector primers M13 P4-22 (5'-CCAGGGTTTCCAGTCACGAC-3', SEQ ID NO: 11) and M13 P5-22 (5'-TCACACAGGAAACAGCTATGAC-3', SEQ ID NO: 12). After the completion of PCR, a DNA insert thus amplified was separated from unreacted primers and nucleotides by gel filtration, etc. to serve as a template for sequencing.

Example 3 Northern analysis

Distribution of human OCTN1 in tissues was investigated by Northern analysis (Fig. 2). A 3'-end fragment of human OCTN1 (the latter half from around the base 1,100) was labelled with [α -³²P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to serve as a probe. Hybridization was performed using the Multiple Tissue Northern (MTN) Blot - Human, Human III, Human IV, Human Fetal II, and Human Cell lines (CLONTECH) at 68°C in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final

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washing was performed at 50°C in 0.1 x SSC and 0.1% SDS. As a result, RNA of about 2.5 kb was strongly expressed in the fetal liver and adult-derived tissues such as the kidney, bone marrow, and trachea. Besides those tissues, the RNA band was also weakly detected in the fetal kidney and lung, and adult tissues including skeletal muscle, lung, placenta, prostate, spleen, and spinal cord. The RNA expression was also detected in tumor cell lines such as HeLa S3, K562, SW480, and A549, and especially, its very intense expression was observed in HeLa S3.

10 Example 4 Cloning of human OCTN2 cDNA

Data base search using the entire nucleotide sequence of "human OCTN1" detected very similar sequences thereto in several parts of the nucleotide sequence of P1 phage clones (P1 H24 clones, GenBank accession No. L43407, L43408, L46907, L81773, and L43409) derived from q regions of human chromosome 5. The parts having similarity with the nucleotide sequence of human OCTN1 are separated by the sequences having no similarity to the human OCTN1 sequence. The sequence obtained by connecting these similar parts with each other with reference to the sequence of human OCTN1 has a high homology over a wide range with human OCTN1, indicating the presence of OCTN1 homologues. The genomic sequence registered in data base was an incomplete one without covering the entire coding region, and, from only this sequence, it was impossible to know the complete structure of a protein partially encoded by the sequence. Therefore, cDNA cloning of this OCTN1 homologous gene (OCTN2) was performed to determine the coded protein structure. First, 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631R A1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) were prepared based on sequences of these P1 phage clones. PCR was performed using a set of these primers and cDNA synthesized from poly(A)⁺ RNA derived from the human adult kidney (CLONTECH) as a template, under the following conditions: 1 cycle of 94°C for 3 min; 35 cycles of 94°C for 30 s, 58°C for 1 min, and 72°C for 2 min,; and 1 cycle of 72°C for 10 min, resulting in amplification of about 900 bp fragment. This fragment was subcloned into the pT7Blue-T vector

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(Novagen) by the TA cloning method to determine its nucleotide sequence, which clearly showed a very high overall homology with human OCTN1. Therefore, this gene was designated as human OCTN2, and longer cDNAs were cloned.

5 The cDNA library derived from the human kidney was screened using the cDNA insert of this clone as a probe in the same manner as for human OCTN1 cDNA cloning, and cDNA containing the entire coding region of human OCTN2 was cloned by a procedure for isolating longer clone and the Rapid Amplification of cDNA Ends (RACE) method
10 (Chenchik, A., Mogadam, F., and Siebert, P. (1995), CLONTECHniques X, 5-8), etc. to determine its structure (SEQ ID NO: 4). Specifically, the RACE method was carried out as follows. The 631R S6 primer (5'-AGCATCCTGTCTCCCTACTTCGTT-3', SEQ ID NO: 15) was prepared. PCR was performed using this primer and the
15 Marathon-Ready™ cDNA derived from the human adult kidney (CLONTECH) as a template under the following conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 3 min,; and 72°C for 10 min, resulting in amplification of about 1.7 kbp cDNA fragment of the 3'-end. This fragment was subcloned into
20 the pT7Blue-T vector by the TA cloning method to determine its structure.

 It became evident that human OCTN2 contains an open reading frame (ORF) encoding a protein consisting of 557 amino acid residues. Fig. 3 compares amino acid sequences of human OCTN1
25 and human OCTN2. Both showed overall amino acid homology as high as about 76%. In addition, one consensus sequence (160 to 176) of sugar transporter was present in the amino acid sequence of human OCTN2 like human OCTN1. These facts indicated that human OCTN2 can be a novel transporter that is structurally related to
30 human OCTN1. Furthermore, a consensus sequence (218 to 225) of the ATP/GTP binding site was also present in the amino acid sequence of human OCTN2 like in human OCTN1.

Example 5 Northern analysis

 Northern analysis was performed using about 900 bp human OCTN2
35 cDNA as a probe which was obtained by PCR with a set of 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTC-3', SEQ ID NO: 13) and 631R

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Al primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) in the same manner as for human OCTN1. The results are shown in Fig. 4. Although the expression pattern of human OCTN2 partly overlapped with that of human OCTN1, human OCTN2 differs from human OCTN1 in that the former was very intensely expressed in the kidney among fetal tissues, while the latter was strongly expressed also in cancer cell strains such as K-562, HeLa S3, SW480, etc. as well as the kidney, indicating that OCTN1 and OCTN2 may be involved in transport of substances such as carcinostatics in these cancer cells.

Example 6 Forced expression of human OCTN1 in human fetal kidney cells (HEK293) and its activity determination

Phage DNAs were extracted from positive phage clones obtained by screening the clones by the plaque hybridization method using the QIAGEN Lambda Kit (QIAGEN). After the DNA insert was subcloned into the pUC18 vector, cDNA containing the entire ORF which was cleaved out with SmaI and EcoRI was integrated between the EcoRI site and the blunted HindIII site of an expression vector for mammalian cells, pCDNA3 (Invitrogen), to obtain an expression plasmid DNA, pCDNA3/OCTN1. Plasmid DNA was prepared by alkaline-SDS method using the QIAGEN PLASMID MAXI Kit (QIAGEN).

The human fetal kidney-derived cell strain, HEK 293 cells were transfected with the plasmid pCDNA3/OCTN1 and pCDNA3 vector containing no insert as a control by the calcium phosphate method. First, the plasmid DNA (10 μ g), a Hepes buffer solution (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4 , 6 mM Dextrose, and 21 mM Hepes pH 7.1) (1 ml), and 2M CaCl_2 (62.5 μ l) were combined and allowed to stand at room temperature for 30 min or more to form calcium phosphate coprecipitates. After cells were plated on 10-cm plates at 1.5×10^6 cells per plate and cultured for 24 h, the calcium phosphate coprecipitates were added thereto, and the cells were further cultured for 24 h. Then, plates were washed with phosphate buffered saline (PBS), and the cells were further cultured for 24 h after the addition of fresh culture medium.

Transport experiment was performed using cells transfected with the plasmid DNA or untreated cells according to the following

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procedures. Cells were detached from plates using a rubber policeman, suspended in a transport buffer (containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM (+)-glucose, 1.2 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, and 25 mM Hepes pH 7.4), and pre-incubated for 20 min.

5 An appropriate amount of each labeled substrate ([¹⁴C]TEA (tetraethylammonium) (NEN), [³H]carnitine (L-carnitine hydrochloride) (Amersham), [³H]PCG (benzylpenicillin) (Amersham), [³H]quinidine (ARC), or [³H]pyrilamine (mepyramine) (Amersham)) was then added to the cell suspension, and the resulting mixture

10 was incubated at 37°C for a predetermined period of time. Incubated cells were overlaid on a silicon layer prepared by layering a mixture of silicon oil and liquid paraffin (specific gravity = 1.022) on a 3 M KCl layer, and separated by centrifugation. Radioactivity of cells was measured to determine the into-the-cell

15 transport activity. In this case, 1×10^6 cells were used as one point of cells. HEK 293 cells were cultured in Dulbecco's MEM containing 10% fetal calf serum (FCS) in an atmosphere of 5% carbon dioxide at 37°C.

First, the transporter capacity was measured in the cells

20 transfected with pcDNA3/OCTN1 and untreated cells using TEA as a substrate (Fig. 5). A reaction time-dependent TEA uptake into the human OCTN1-transfected cells was clearly observed. This uptake was not observed in untreated cells. Next, effects of the addition of unlabeled TEA on the labeled substrate uptake in this

25 system (cold inhibition) was examined (Fig. 6). A decrease in the apparent uptake of the labeled substrate was clearly seen depending on the concentration of cold TEA added. In this experiment, almost no uptake of the substrate into cells was observed in cells transfected with the pcDNA3 vector containing

30 no insert (Mock) used as a control like in untreated cells used, clearly indicating that this uptake phenomenon is due to the transfection of the cells with human OCTN1. Next, to obtain the K_m (Michaelis constant) value of human OCTN1 to TEA, the uptake of ¹⁴C-TEA with various concentrations was measured (Fig. 7). From

35 Lineweaver-Burk reciprocal plot of the net uptake obtained by subtracting the amount of the uptake in Mock cells from that in the human OCTN1-transfected cells, the K_m value of 0.44 ± 0.04 mM

was obtained with the maximal velocity, V_{max} of 6.68 ± 0.34 (nmol/3 min/mg). Next, the transport capacity of human OCTN1 for other substrate than TEA was examined (Fig. 8). When the transport capacity was measured using labeled organic cations such as
5 labeled carnitine, quinidine, and pyrilamine, a significant increase in the uptake of these compounds was clearly observed in human OCTN1-transfected cells as compared with Mock cells, clearly indicating that these organic cations can serve as substrates for human OCTN1. However, no significant increase in
10 the uptake of an organic anion, PCG (benzylpenicillin), was observed.

Example 7 Activity measurement of human OCTN1 using *Xenopus* oocytes

crRNA was synthesized *in vitro* using T7 RNA polymerase with
15 pcDNA3/OCTN1 as a template. This crRNA was diluted to the concentration of 0.3 ng/nl, and its 50-nl (15 ng) aliquot was injected into a single oocyte. As a control, 50 nl of distilled water was injected. These oocytes were cultured for 3 days, and then used for the transport experiment. After being preincubated
20 in an uptake buffer (0.05% Tween 80, 100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes pH 7.4) at 25°C for 20 min, the oocytes were transferred to the uptake buffer containing 0.5 ml of labeled substrate to initiate the uptake. After the incubation at 25°C for 1 h, the oocytes were washed in the ice-cold uptake
25 buffer three times to terminate the reaction. The oocytes were solubilized in 5% SDS and mixed with Cleasol I (a cocktail for liquid scintillation counter) (3 ml) to determine the radioactivity. The radioactivity of the uptake buffer which contained the labeled compound at the time of incubation (external
30 solution) (10 μ l) was also similarly measured. The ratio of the radioactivity (dpm value) in the oocytes to that (dpm value) in the external solution was used as the uptake activity.

Human OCTN1 also expresses the transport capacity for organic cations such as quinidine, mepyramine and carnitine, as well as
35 TEA in this transport experiment system using *Xenopus* oocytes (Fig. 9).

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Next, the transport capacity of human OCTN1 for carcinostatics, etc. was examined. The results revealed that human OCTN1 has the activity to transport actinomycin D, etoposide, vinblastine, and daunomycin (Fig. 10). These results strongly indicate that OCTN1 would be involved in the into-the-cell translocation mechanism (mechanism for absorption by cells) for these drugs, which have been clinically used as carcinostatics. By designing and screening drugs utilizing the substrate specificity of OCTN1 so as to be readily recognized by this transporter, it would be possible to efficiently develop useful drugs that can be readily absorbed by the cells.

Example 8 Forced expression of human OCTN2 in HEK cells and its activity measurement

The expression plasmid DNA for human OCTN2 in mammalian cells was prepared as follows.

A single-stranded cDNA was synthesized from poly(A)⁺ RNA derived from the human fetal kidney (CLONTECH) using the SuperScriptTM II reverse transcriptase (GIBCO BRL). PCR was performed using the thus-obtained cDNA as a template under the following conditions to amplify 5'- and 3'-end fragments of human OCTN2.

For the amplification of 5'-end fragment (about 800 bp) of human OCTN2, OCTN2 3 primer (5'-GATGGATCCCGGACGGTCTTGGGTCGCCTGCTG-3', SEQ ID NO: 16) and OCTN2 4 primer (5'-GATGGATCCAAATGCTGCCACATAGTTGGAGAT-3', SEQ ID NO: 17) were used. PCR was carried out using DNA polymerase ExTaq (TaKaRa) and dNTPs (150 μ M 7-deaza dGTP, 50 μ M dGTP, 200 μ M dATP, 200 μ M dTTP, and 200 μ M dCTP) according to the following conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 63°C for 1 min, and 72°C for 2 min; and 72°C for 10 min. For the amplification of 3'-end fragment (about 1.2 kbp) of human OCTN2, OCTN2 7 primer (5'-GATGGATCCATGGGCATGCAGACAGGCTTCAGC-3', SEQ ID NO: 18) and OCTN2 8 primer (5'-GATGGATCCTTCCTCTTCAGTTTCTCCCTTACT-3', SEQ ID NO: 19) were used. PCR was carried out using DNA polymerase ExTaq (TaKaRa) and dNTPs (200 μ M dGTP, 200 μ M dATP, 200 μ M dTTP, and 200 μ M dCTP) according to the following conditions: 94°C for 2 min;

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35 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 2 min,; and 72°C for 10 min.

These fragments were respectively electrophoresed on agarose gel, excised from the gel, purified, and subcloned into the pT7Blue-T vector. Clones having no PCR error were selected by sequencing, and clones from both fragments were ligated at the PstI site in the overlapping region. Each ligated fragment was eventually incorporated into the BamHI site of the pcDNA3 vector, and used as the expression plasmid DNA pcDNA3/OCTN2.

HEK cells were transfected with pcDNA3/OCTN2, the pcDNA3 vector containing no insert (Mock), or pcDNA3/OCTN1 by the method described in Example 6 to perform transport experiments. It was proved that human OCTN2 has a high capacity to efficiently transport carnitine (Fig. 11). On the other hand, human OCTN2 hardly transported TEA, which were efficiently transported by human OCTN1, revealing that they clearly differ in their substrate specificities.

Next, Na⁺ dependence of human OCTN2-mediated carnitine transport was examined using a transport buffer in which Na⁺ was replaced with K⁺ (Fig. 12). The result showed that carnitine transport mediated by human OCTN2 completely depended on the presence of Na⁺, indicating that OCTN2 is a symport type transporter that transports substrates and Na⁺ in the same direction.

Example 9 Cloning of mouse OCTN1

Data base search using human OCTN1 cDNA sequence detected several Expressed Sequence Tags (ESTs) derived from mouse, which had very high homology to the human OCTN1 cDNA sequence. Based on these EST sequences, MONL 1 primer (5'-CGCGCCGAATCGCTGAATCCTTTC-3', SEQ ID NO: 20) and MONA 4 primer (5'-AGGCTTTTGATTTGTTCTGTTGAG-3', SEQ ID NO: 21) were prepared. PCR was performed using a set of these primers and cDNA prepared from poly(A)⁺ RNA derived from the mouse kidney as a template. As a result, fragments of about 2 kbp were amplified. These fragments were electrophoresed on agarose gels, excised from the gels,

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purified, and subcloned into the pT7Blue T vector (Novagen) by the TA cloning method. The sequence of mouse OCTN1 was determined by sequencing plural clones. The nucleotide sequence of cDNA thus determined is shown in SEQ ID NO: 23, and amino acid sequence of
 5 the protein encoded by the cDNA in SEQ ID NO: 22.

Example 10 Cloning of mouse OCTN2

First, MONB 20 primer (5'-CCCATGCCAACAAGGACAAAAGC-3', SEQ ID NO: 24) was prepared from the sequence of human OCTN2 cDNA. The Marathon-ReadyTM cDNA derived from the mouse kidney (CLONTECH)
 10 was used as a template for the 5'-Rapid Amplification of cDNA ends (RACE) to clone the 5'-end sequence upstream of the primer. Next, data base search was performed using human OCTN2 nucleotide sequence to detect several ESTs derived from mouse, which had a very high homology with human OCTN2. MONB 26 primer (5'-
 15 ACAGAACAGAAAAGCCCTCAGTCA-3', SEQ ID NO: 25) was prepared from these EST sequences. MONB 6 primer (5'-TGTTTTTCGTGGGTGTGCTGATGG-3', SEQ ID NO: 26) was prepared from the sequence obtained by the 5'-RACE. PCR was performed using this primer and MONB 26 primer and cDNA prepared from poly(A)⁺ RNA
 20 derived from the mouse kidney as a template to amplify the 3'-end fragments. The sequence of mouse OCTN2 was determined by sequencing directly of after subcloning respective fragments. The nucleotide sequence of the cDNA thus determined is shown in SEQ ID NO: 28, and amino acid sequence of the protein encoded by
 25 the cDNA in SEQ ID NO: 27.

Example 11 Tissue expression analysis of mouse OCTN1 and mouse OCTN2

The expression amount of mouse OCTN1 and mouse OCTN2 genes in various tissues was examined by RT-PCR using a mouse Multiple
 30 Tissue cDNA (MTC) panel (CLONTECH) (Fig. 13). Primers used are MONL 1 and MONA 4 for mouse OCTN1, and MONB 6 and MONB 26 for mouse OCTN2. As a result, the high level expression of mouse OCTN1 was detected in the kidney and liver, while that of mouse OCTN2 in the kidney, liver, and 7-days old embryo.

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Industrial Applicability

This invention provides a family of novel organic cation transporter genes and proteins encoded by these genes. Transporter proteins of this invention are useful for developing
5 newly designed drugs that can be transported mediated by these proteins, and pharmaceuticals for disorders caused by functional abnormalities of the proteins.

SEQUENCE LISTING

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Arg Trp Leu Ile Ser Gln Arg Arg Phe Arg Glu Ala Glu Asp Ile Ile	
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caa aaa gct gca aaa atg aac aac aca gct gta cca gca gtg ata ttt	1085
Gln Lys Ala Ala Lys Met Asn Asn Thr Ala Val Pro Ala Val Ile Phe	
300 305 310	
gat tct gtg gag gag cta aat ccc ctg aag cag cag aaa gct ttc att	1133
Asp Ser Val Glu Glu Leu Asn Pro Leu Lys Gln Gln Lys Ala Phe Ile	
315 320 325	
ctg gac ctg ttc agg act cgg aat att gcc ata atg acc att atg tct	1181
Leu Asp Leu Phe Arg Thr Arg Asn Ile Ala Ile Met Thr Ile Met Ser	
330 335 340 345	
ttg ctg cta tgg atg ctg acc tca gtg ggt tac ttt gct ctg tct ctg	1229
Leu Leu Leu Trp Met Leu Thr Ser Val Gly Tyr Phe Ala Leu Ser Leu	
350 355 360	
gat gct cct aat tta cat gga gat gcc tac ctg aac tgt ttc ctc tct	1277
Asp Ala Pro Asn Leu His Gly Asp Ala Tyr Leu Asn Cys Phe Leu Ser	
365 370 375	
gcc ttg att gaa att cca gct tac att aca gcc tgg ctg cta ttg cga	1325
Ala Leu Ile Glu Ile Pro Ala Tyr Ile Thr Ala Trp Leu Leu Leu Arg	
380 385 390	
acg ctg ccc agg cgt tat atc ata gct gca gta ctg ttc tgg gga gga	1373
Thr Leu Pro Arg Arg Tyr Ile Ile Ala Ala Val Leu Phe Trp Gly Gly	
395 400 405	
ggt gtg ctt ctc ttc att caa ctg gta cct gtg gat tat tac ttc tta	1421
Gly Val Leu Leu Phe Ile Gln Leu Val Pro Val Asp Tyr Tyr Phe Leu	
410 415 420 425	
tcc att ggt ctg gtc atg ctg gga aaa ttt ggg atc acc tct gct ttc	1469
Ser Ile Gly Leu Val Met Leu Gly Lys Phe Gly Ile Thr Ser Ala Phe	
430 435 440	
tcc atg ctg tat gtc ttc act gct gag ctc tac cca acc ctg gtc agg	1517
Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg	
445 450 455	

aac atg gcg gtg ggg gtc aca tcc acg gcc tcc aga gtg ggc agc atc 1565
 Asn Met Ala Val Gly Val Thr Ser Thr Ala Ser Arg Val Gly Ser Ile
 460 465 470

att gcc ccc tac ttt gtt tac ctc ggt gct tac aac aga atg ctg ccc 1613
 Ile Ala Pro Tyr Phe Val Tyr Leu Gly Ala Tyr Asn Arg Met Leu Pro
 475 480 485

tac atc gtc atg ggt agt ctg act gtc ctg att gga atc ttc acc ctt 1661
 Tyr Ile Val Met Gly Ser Leu Thr Val Leu Ile Gly Ile Phe Thr Leu
 490 495 500 505

ttt ttc cct gaa agt ttg gga atg act ctt cca gaa acc tta gag cag 1709
 Phe Phe Pro Glu Ser Leu Gly Met Thr Leu Pro Glu Thr Leu Glu Gln
 510 515 520

atg cag aaa gtg aaa tgg ttc aga tct ggg aaa aaa aca aga gac tca 1757
 Met Gln Lys Val Lys Trp Phe Arg Ser Gly Lys Lys Thr Arg Asp Ser
 525 530 535

atg gag aca gaa gaa aat ccc aag gtt cta ata act gca ttc 1799
 Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
 540 545 550

tgaaaaaata tctaccccat ttggtgaagt gaaaaacaga aaaataagac cctgtggaga 1859

aattcgttgt tcccactgaa atggactgac tgtaacgatt gacaccaaaa tgaaccttgc 1919

tatcaagaaa tgctcgtcat acagtaaact ctggatgatt cttccagata atgtccttgc 1979

ttacaaacc aaccatttct agagagtctc ctactcatt aattcaatga aatggattgg 2039

taagatgtct tgaaaacatg ttagtcaagg actggtaaaa tacatataaa gattaacact 2099

catttccaat catacaaata ctatccaaat aaaaat 2135

<210> 3

<211> 557

<212> PRT

<213> Homo sapiens

<400> 3

Met	Arg	Asp	Tyr	Asp	Glu	Val	Thr	Ala	Phe	Leu	Gly	Glu	Trp	Gly	1	5	10	15	
Pro	Phe	Gln	Arg	Leu	Ile	Phe	Phe	Leu	Leu	Ser	Ala	Ser	Ile	Ile	Pro	20	25	30	
Asn	Gly	Phe	Thr	Gly	Leu	Ser	Ser	Val	Phe	Leu	Ile	Ala	Thr	Pro	Glu	35	40	45	
His	Arg	Cys	Arg	Val	Pro	Asp	Ala	Ala	Asn	Leu	Ser	Ser	Ala	Trp	Arg	50	55	60	
Asn	His	Thr	Val	Pro	Leu	Arg	Leu	Arg	Asp	Gly	Arg	Glu	Val	Pro	His	65	70	75	
Ser	Cys	Arg	Arg	Tyr	Arg	Leu	Ala	Thr	Ile	Ala	Asn	Phe	Ser	Ala	Leu	80	85	90	95
Gly	Leu	Glu	Pro	Gly	Arg	Asp	Val	Asp	Leu	Gly	Gln	Leu	Glu	Gln	Glu	100	105	110	
Ser	Cys	Leu	Asp	Gly	Trp	Glu	Phe	Ser	Gln	Asp	Val	Tyr	Leu	Ser	Thr	115	120	125	
Ile	Val	Thr	Glu	Trp	Asn	Leu	Val	Cys	Glu	Asp	Asp	Trp	Lys	Ala	Pro	130	135	140	
Leu	Thr	Ile	Ser	Leu	Phe	Phe	Val	Gly	Val	Leu	Leu	Gly	Ser	Phe	Ile	145	150	155	
Ser	Gly	Gln	Leu	Ser	Asp	Arg	Phe	Gly	Arg	Lys	Asn	Val	Leu	Phe	Val	160	165	170	175
Thr	Met	Gly	Met	Gln	Thr	Gly	Phe	Ser	Phe	Leu	Gln	Ile	Phe	Ser	Lys	180	185	190	
Asn	Phe	Glu	Met	Phe	Val	Val	Leu	Phe	Val	Leu	Val	Gly	Met	Gly	Gln	195	200	205	
Ile	Ser	Asn	Tyr	Val	Ala	Ala	Phe	Val	Leu	Gly	Thr	Glu	Ile	Leu	Gly	210	215	220	
Lys	Ser	Val	Arg	Ile	Ile	Phe	Ser	Thr	Leu	Gly	Val	Cys	Ile	Phe	Tyr				

225		230		235
Ala Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp				
240		245		255
Trp Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val				
	260		265	270
Ala Leu Trp Trp Phe Ile Pro Glu Ser Pro Arg Trp Leu Ile Ser Gln				
	275		280	285
Gly Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala				
	290		295	300
Asn Gly Ile Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln				
	305		310	315
Asp Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu				
	320		325	330
Arg Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp				
	340		345	350
Met Thr Ile Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn				
	355		360	365
Leu His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu				
	370		375	380
Val Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg				
	385		390	395
Arg Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu				
	400		405	410
Phe Met Gln Leu Val Pro Pro Asp Leu Tyr Tyr Leu Ala Thr Val Leu				
	420		425	430
Val Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr				
	435		440	445
Val Tyr Thr Ala Glu Leu Tyr Pro Thr Val Val Arg Asn Met Gly Val				
	450		455	460

10

Gly Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr
 465 470 475

Phe Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met
 480 485 490 495

Gly Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu
 500 505 510

Ser Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val
 515 520 525

Lys Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys
 530 535 540

Asp Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe
 545 550 555

<210> 4

<211> 1831

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (124)..(1794)

<400> 4

cggacgggtct tgggtcgcct gctgcctggc ttgcctggtc ggcgggcgggt gccccgcgcg 60

cacgcgcaaa gccccgcgcg ttcccagacc ccaggccgcg ctctgtgggc ctctgagggc 120

ggc atg cgg gac tac gac gag gtg acc gcc ttc ctg ggc gag tgg ggg 168
 Met Arg Asp Tyr Asp Glu Val Thr Ala Phe Leu Gly Glu Trp Gly
 1 5 10 15

ccc ttc cag cgc ctc atc ttc ttc ctg ctc agc gcc agc atc atc ccc 216
 Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu Ser Ala Ser Ile Ile Pro
 20 25 30

aat ggc ttc acc ggc ctg tcc tcc gtg ttc ctg ata gcg acc ccg gag 264
 Asn Gly Phe Thr Gly Leu Ser Ser Val Phe Leu Ile Ala Thr Pro Glu

35					40					45						
cac	cgc	tgc	egg	gtg	ccg	gac	gcc	gcg	aac	ctg	agc	agc	gcc	tgg	cgc	312
His	Arg	Cys	Arg	Val	Pro	Asp	Ala	Ala	Asn	Leu	Ser	Ser	Ala	Trp	Arg	
		50					55					60				
aac	cac	act	gtc	cca	ctg	cgg	ctg	cgg	gac	ggc	cgc	gag	gtg	ccc	cac	360
Asn	His	Thr	Val	Pro	Leu	Arg	Leu	Arg	Asp	Gly	Arg	Glu	Val	Pro	His	
	65					70					75					
agc	tgc	cgc	cgc	tac	cgg	ctc	gcc	acc	atc	gcc	aac	ttc	tcg	gcg	ctc	408
Ser	Cys	Arg	Arg	Tyr	Arg	Leu	Ala	Thr	Ile	Ala	Asn	Phe	Ser	Ala	Leu	
80					85					90					95	
ggg	ctg	gag	ccg	ggg	cgc	gac	gtg	gac	ctg	ggg	cag	ctg	gag	cag	gag	456
Gly	Leu	Glu	Pro	Gly	Arg	Asp	Val	Asp	Leu	Gly	Gln	Leu	Glu	Gln	Glu	
			100						105					110		
agc	tgt	ctg	gat	ggc	tgg	gag	ttc	agt	cag	gac	gtc	tac	ctg	tcc	acc	504
Ser	Cys	Leu	Asp	Gly	Trp	Glu	Phe	Ser	Gln	Asp	Val	Tyr	Leu	Ser	Thr	
			115					120					125			
att	gtg	acc	gag	tgg	aac	ctg	gtg	tgt	gag	gac	gac	tgg	aag	gcc	cca	552
Ile	Val	Thr	Glu	Trp	Asn	Leu	Val	Cys	Glu	Asp	Asp	Trp	Lys	Ala	Pro	
		130					135					140				
ctc	aca	atc	tcc	ttg	ttc	ttc	gtg	ggt	gtg	ctg	ttg	ggc	tcc	ttc	att	600
Leu	Thr	Ile	Ser	Leu	Phe	Phe	Val	Gly	Val	Leu	Leu	Gly	Ser	Phe	Ile	
	145					150					155					
tca	ggg	cag	ctg	tca	gac	agg	ttt	ggc	cgg	aag	aat	gtg	ctg	ttc	gtg	648
Ser	Gly	Gln	Leu	Ser	Asp	Arg	Phe	Gly	Arg	Lys	Asn	Val	Leu	Phe	Val	
160					165				170					175		
acc	atg	ggc	atg	cag	aca	ggc	ttc	agc	ttc	ctg	cag	atc	ttc	tcg	aag	696
Thr	Met	Gly	Met	Gln	Thr	Gly	Phe	Ser	Phe	Leu	Gln	Ile	Phe	Ser	Lys	
				180				185					190			
aat	ttt	gag	atg	ttt	gtc	gtg	ctg	ttt	gtc	ctt	gta	ggc	atg	ggc	cag	744
Asn	Phe	Glu	Met	Phe	Val	Val	Leu	Phe	Val	Leu	Val	Gly	Met	Gly	Gln	
		195					200					205				
atc	tcc	aac	tat	gtg	gca	gca	ttt	gtc	ctg	ggg	aca	gaa	att	ctt	ggc	792

Ile Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly	
210 215 220	
aag tca gtt cgt ata ata ttc tct acg tta gga gtg tgc ata ttt tat	840
Lys Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr	
225 230 235	
gca ttt ggc tac atg gtg ctg cca ctg ttt gct tac ttc atc cga gac	888
Ala Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp	
240 245 250 255	
tgg cgg atg ctg ctg gtg gcg ctg acg atg ccg ggg gtg ctg tgc gtg	936
Trp Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val	
260 265 270	
gca ctc tgg tgg ttc atc cct gag tcc ccc cga tgg ctc atc tct cag	984
Ala Leu Trp Trp Phe Ile Pro Glu Ser Pro Arg Trp Leu Ile Ser Gln	
275 280 285	
gga cga ttt gaa gag gca gag gtg atc atc cgc aag gct gcc aaa gcc	1032
Gly Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala	
290 295 300	
aat ggg att gtt gtg cct tcc act atc ttt gac ccg agt gag tta caa	1080
Asn Gly Ile Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln	
305 310 315	
gac cta agt tcc aag aag cag cag tcc cac aac att ctg gat ctg ctt	1128
Asp Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu	
320 325 330 335	
cga acc tgg aat atc cgg atg gtc acc atc atg tcc ata atg ctg tgg	1176
Arg Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp	
340 345 350	
atg acc ata tca gtg ggc tat ttt ggg ctt tcg ctt gat act cct aac	1224
Met Thr Ile Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn	
355 360 365	
ttg cat ggg gac atc ttt gtg aac tgc ttc ctt tca gcg atg gtt gaa	1272
Leu His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu	
370 375 380	

gtc cca gca tat gtg ttg gcc tgg ctg ctg ctg caa tat ttg ccc cgg	1320
Val Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg	
385 390 395	
cgc tat tcc atg gcc act gcc ctc ttc ctg ggt ggc agt gtc ctt ctc	1368
Arg Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu	
400 405 410 415	
ttc atg cag ctg gta ccc cca gac ttg tat tat ttg gct aca gtc ctg	1416
Phe Met Gln Leu Val Pro Pro Asp Leu Tyr Tyr Leu Ala Thr Val Leu	
420 425 430	
gtg atg gtg ggc aag ttt gga gtc acg gct gcc ttt tcc atg gtc tac	1464
Val Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr	
435 440 445	
gtg tac aca gcc gag ctg tat ccc aca gtg gtg aga aac atg ggt gtg	1512
Val Tyr Thr Ala Glu Leu Tyr Pro Thr Val Val Arg Asn Met Gly Val	
450 455 460	
gga gtc agc tcc aca gca tcc cgc ctg ggc agc atc ctg tct ccc tac	1560
Gly Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr	
465 470 475	
ttc gtt tac ctt ggt gcc tac gac cgc ttc ctg ccc tac att ctc atg	1608
Phe Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met	
480 485 490 495	
gga agt ctg acc atc ctg aca gcc atc ctc acc ttg ttt ctc cca gag	1656
Gly Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu	
500 505 510	
agc ttc ggt acc cca ctc cca gac acc att gac cag atg cta aga gtc	1704
Ser Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val	
515 520 525	
aaa gga atg aaa cac aga aaa act cca agt cac aca agg atg tta aaa	1752
Lys Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys	
530 535 540	
gat ggt caa gaa agg ccc aca atc ctt aaa agc aca gcc ttc	1794
Asp Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe	
545 550 555	

taacatcgct tccagtaagg gagaaactga agaggaa

1831

<210> 5

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 5

ctaatacgac tcactatagg gc

22

<210> 6

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 6

tgtagcgtga agacgacaga a

21

<210> 7

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 7

tcgagcggcc gcccgggcag gt

22

<210> 8

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 8

agggcgtggt gcggagggcg gt

22

<210> 9

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 9

cttttgagca agttcagcct

20

<210> 10

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 10

agaggtggct tatgagtatt tctt

24

<210> 11

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 11

ccagggtttt cccagtcacg ac

22

<210> 12
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 12
tcacacagga aacagctatg ac 22

<210> 13
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 13
gtgctgttgg gctccttcat ttca 24

<210> 14
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 14
agctgcatga agagaaggac actg 24

<210> 15
<211> 24
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 15

agcatcctgt ctccctactt cggt

24

<210> 16

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 16

gatggatccc ggacggtctt gggtcgcctg ctg

33

<210> 17

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 17

gatggatcca aatgctgcca catagttgga gat

33

<210> 18

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 18

gatggatcca tgggcatgca gacaggcttc agc

33

<210> 19

<211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 19
 gatggatcct tcctcttcag tttctccctt act 33

<210> 20
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 20
 cgcgccgaat cgctgaatcc tttc 24

<210> 21
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Artificial Synthesized Primer Sequence

<400> 21
 aggcttttga tttgttctgt tgag 24

<210> 22
 <211> 553
 <212> PRT
 <213> Mus musculus

<400> 22
 Met Arg Asp Tyr Asp Glu Val Ile Ala Phe Leu Gly Glu Trp Gly Pro

19

1	5	10	15
Phe Gln Arg Leu Ile Phe Phe Leu Leu Ser Ala Ser Ile Ile Pro Asn	20	25	30
Gly Phe Asn Gly Met Ser Val Val Phe Leu Ala Gly Thr Pro Glu His	35	40	45
Arg Cys Leu Val Pro Asp Thr Val Asn Leu Ser Ser Ser Trp Arg Asn	50	55	60
His Ser Ile Pro Leu Glu Thr Lys Asp Gly Arg Gln Val Pro Gln Ser	65	70	75
Cys Arg Arg Tyr Arg Leu Ala Thr Ile Ala Asn Phe Ser Ala Met Gly	85	90	95
Leu Glu Pro Gly Gln Asp Val Asp Leu Glu Gln Leu Glu Gln Glu Ser	100	105	110
Cys Leu Asp Gly Trp Glu Tyr Asp Lys Asp Ile Phe Leu Ser Thr Ile	115	120	125
Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Thr Pro Leu	130	135	140
Thr Thr Ser Leu Phe Phe Val Gly Val Leu Cys Gly Ser Phe Val Ser	145	150	155
Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Lys Val Leu Phe Ala Thr	165	170	175
Met Ala Val Gln Thr Gly Phe Ser Phe Val Gln Ile Phe Ser Thr Asn	180	185	190
Trp Glu Met Phe Thr Val Leu Phe Ala Ile Val Gly Met Gly Gln Ile	195	200	205
Ser Asn Tyr Val Val Ala Phe Ile Leu Gly Thr Glu Ile Leu Ser Lys	210	215	220
Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Thr Phe Phe Ala	225	230	235
			240

Ile	Gly	Tyr	Met	Val	Leu	Pro	Leu	Phe	Ala	Tyr	Phe	Ile	Arg	Asp	Trp	
				245					250					255		
Arg	Met	Leu	Leu	Leu	Ala	Leu	Thr	Leu	Pro	Gly	Leu	Phe	Cys	Val	Pro	
			260					265					270			
Leu	Trp	Trp	Phe	Ile	Pro	Glu	Ser	Pro	Arg	Trp	Leu	Ile	Ser	Gln	Arg	
		275					280					285				
Arg	Phe	Ala	Glu	Ala	Glu	Gln	Ile	Ile	Gln	Lys	Ala	Ala	Lys	Met	Asn	
	290					295					300					
Ser	Ile	Val	Ala	Pro	Ala	Gly	Ile	Phe	Asp	Pro	Leu	Glu	Leu	Gln	Glu	
305					310					315					320	
Leu	Asn	Ser	Leu	Lys	Gln	Gln	Lys	Val	Ile	Ile	Leu	Asp	Leu	Phe	Arg	
			325						330					335		
Thr	Arg	Asn	Ile	Ala	Thr	Ile	Thr	Val	Met	Ala	Val	Met	Leu	Trp	Met	
			340					345					350			
Leu	Thr	Ser	Val	Gly	Tyr	Phe	Ala	Leu	Ser	Leu	Asn	Val	Pro	Asn	Leu	
		355					360					365				
His	Gly	Asp	Val	Tyr	Leu	Asn	Cys	Phe	Leu	Ser	Gly	Leu	Ile	Glu	Val	
	370					375					380					
Pro	Ala	Tyr	Phe	Thr	Ala	Trp	Leu	Leu	Leu	Arg	Thr	Leu	Pro	Arg	Arg	
385					390					395					400	
Tyr	Ile	Ile	Ala	Gly	Val	Leu	Phe	Trp	Gly	Gly	Gly	Val	Leu	Leu	Leu	
				405					410					415		
Ile	Gln	Val	Val	Pro	Glu	Asp	Tyr	Asn	Phe	Val	Ser	Ile	Gly	Leu	Val	
			420					425					430			
Met	Leu	Gly	Lys	Phe	Gly	Ile	Thr	Ser	Ala	Phe	Ser	Met	Leu	Tyr	Val	
		435					440					445				
Phe	Thr	Ala	Glu	Leu	Tyr	Pro	Thr	Leu	Val	Arg	Asn	Met	Ala	Val	Gly	
	450					455					460					

Ile Thr Ser Met Ala Ser Arg Val Gly Ser Ile Ile Ala Pro Tyr Phe
465 470 475 480

Val Tyr Leu Gly Ala Tyr Asn Arg Leu Leu Pro Tyr Ile Leu Met Gly
485 490 495

Ser Leu Thr Val Leu Ile Gly Ile Ile Thr Leu Phe Phe Pro Glu Ser
500 505 510

Phe Gly Val Thr Leu Pro Glu Asn Leu Glu Gln Met Gln Lys Val Arg
515 520 525

Gly Phe Arg Cys Gly Lys Lys Ser Thr Val Ser Val Asp Arg Glu Glu
530 535 540

Ser Pro Lys Val Leu Ile Thr Ala Phe
545 550

<210> 23

<211> 2083

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (122)..(1780)

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Cys Arg Arg Tyr Arg Leu Ala Thr Ile Ala Asn Phe Ser Ala Met Gly	
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Leu Glu Pro Gly Gln Asp Val Asp Leu Glu Gln Leu Glu Gln Glu Ser	
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Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Lys Val Leu Phe Ala Thr	
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Ile Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp Trp	
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Arg Met Leu Leu Leu Ala Leu Thr Leu Pro Gly Leu Phe Cys Val Pro	
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Thr Arg Asn Ile Ala Thr Ile Thr Val Met Ala Val Met Leu Trp Met	
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Pro Ala Tyr Phe Thr Ala Trp Leu Leu Leu Arg Thr Leu Pro Arg Arg	

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Ile Thr Ser Met Ala Ser Arg Val Gly Ser Ile Ile Ala Pro Tyr Phe				
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Val Tyr Leu Gly Ala Tyr Asn Arg Leu Leu Pro Tyr Ile Leu Met Gly				
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Arg Cys Leu Val Pro His Thr Val Asn Leu Ser Ser Ala Trp Arg Asn
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His Ser Ile Pro Leu Glu Thr Lys Asp Gly Arg Gln Val Pro Gln Lys
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85 90 95

Leu Glu Pro Gly Arg Asp Val Asp Leu Glu Gln Leu Glu Gln Glu Ser
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Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Asn Val Leu Phe Leu Thr
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Met Gly Met Gln Thr Gly Phe Ser Phe Leu Gln Val Phe Ser Val Asn
180 185 190

Phe Glu Met Phe Thr Val Leu Phe Val Leu Val Gly Met Gly Gln Ile
 195 200 205

Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Ser Lys
 210 215 220

Ser Ile Arg Ile Ile Phe Ala Thr Leu Gly Val Cys Ile Phe Tyr Ala
 225 230 235 240

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Leu Asn Ser Thr Lys Pro Gln Leu His His Ile Tyr Asp Leu Ile Arg
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 340 345 350

Thr Ile Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn Leu
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Tyr Ser Ile Ser Ala Ala Leu Phe Leu Gly Gly Ser Val Leu Leu Phe
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28

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Met Val Gly Lys Phe Gly Ile Thr Ser Ala Tyr Ser Met Val Tyr Val
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Cys Arg Arg Tyr Arg Leu Ala Thr Ile Ala Asn Phe Ser Glu Leu Gly	
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Leu Glu Pro Gly Arg Asp Val Asp Leu Glu Gln Leu Glu Gln Glu Ser	
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Cys Leu Asp Gly Trp Glu Tyr Asp Lys Asp Val Phe Leu Ser Thr Ile	
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Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Phe Pro Glu Ser			
500	505	510	
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Phe Gly Val Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val Lys			
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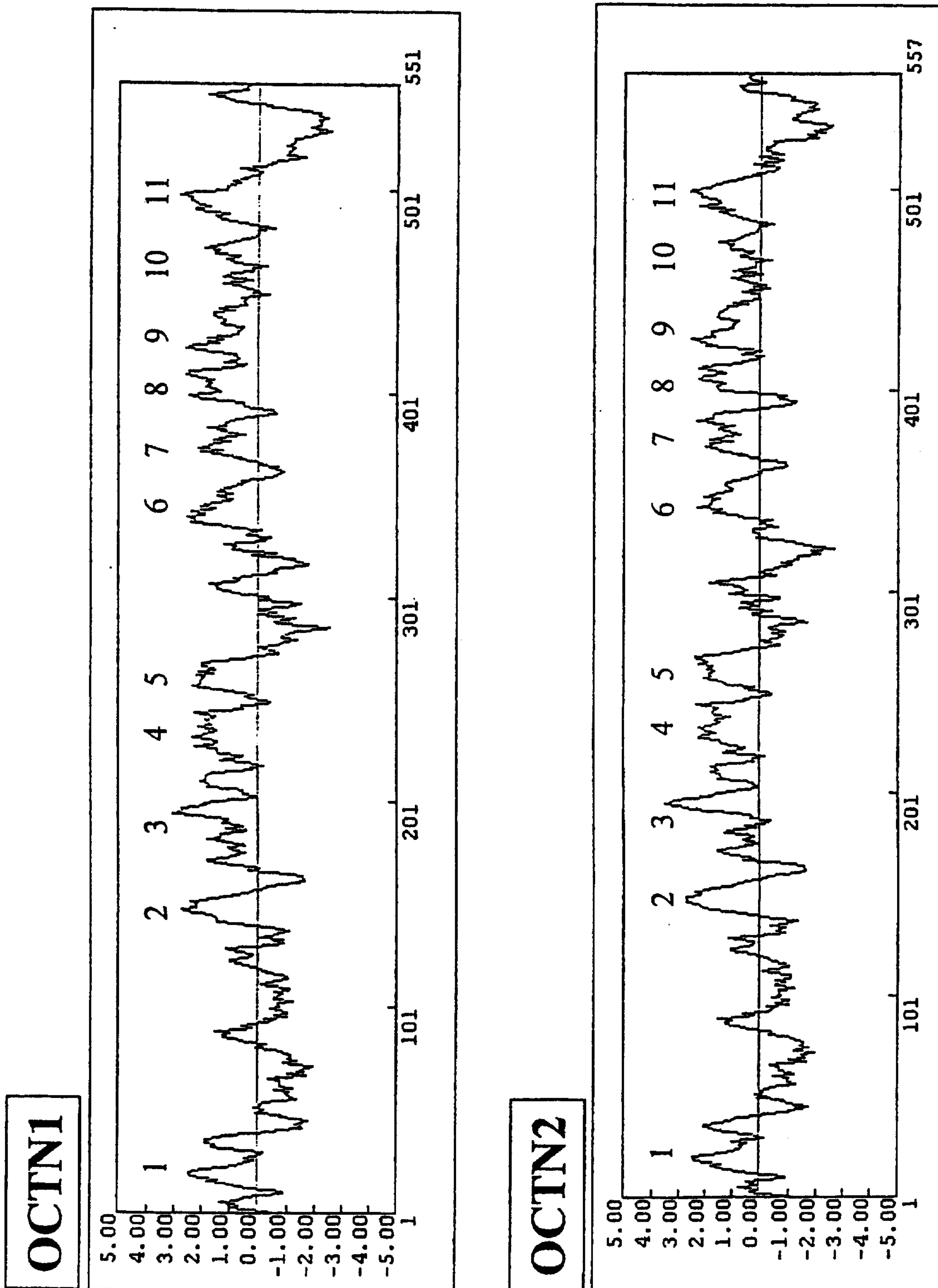
- 26 -

CLAIMS

1. A protein comprising an amino acid sequence set forth in SEQ ID NOs: 1, 3, 22, or 27, or a protein comprising said amino acid
5 sequence in which one or more amino acid residues are substituted, deleted, or added, and having an activity to transport an organic cation.
2. A protein encoded by a DNA hybridizing to a DNA comprising nucleotide sequence set forth in SEQ ID NOs: 2, 4, 23, or 28 and
10 having an activity to transport an organic cation.
3. A DNA encoding the protein according to claim 1 or 2.
4. A vector comprising the DNA according to claim 3.
5. A transformant expressibly carrying the DNA according to claim 3.
- 15 6. A method for producing the protein according to claim 1 or 2, the method comprising culturing the transformant according to claim 5.
7. An antibody that binds to the protein according to claim 1 or 2.
- 20 8. A DNA specifically hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NOs: 2, 4, 23, or 28 and consisting of at least 15 nucleotides.

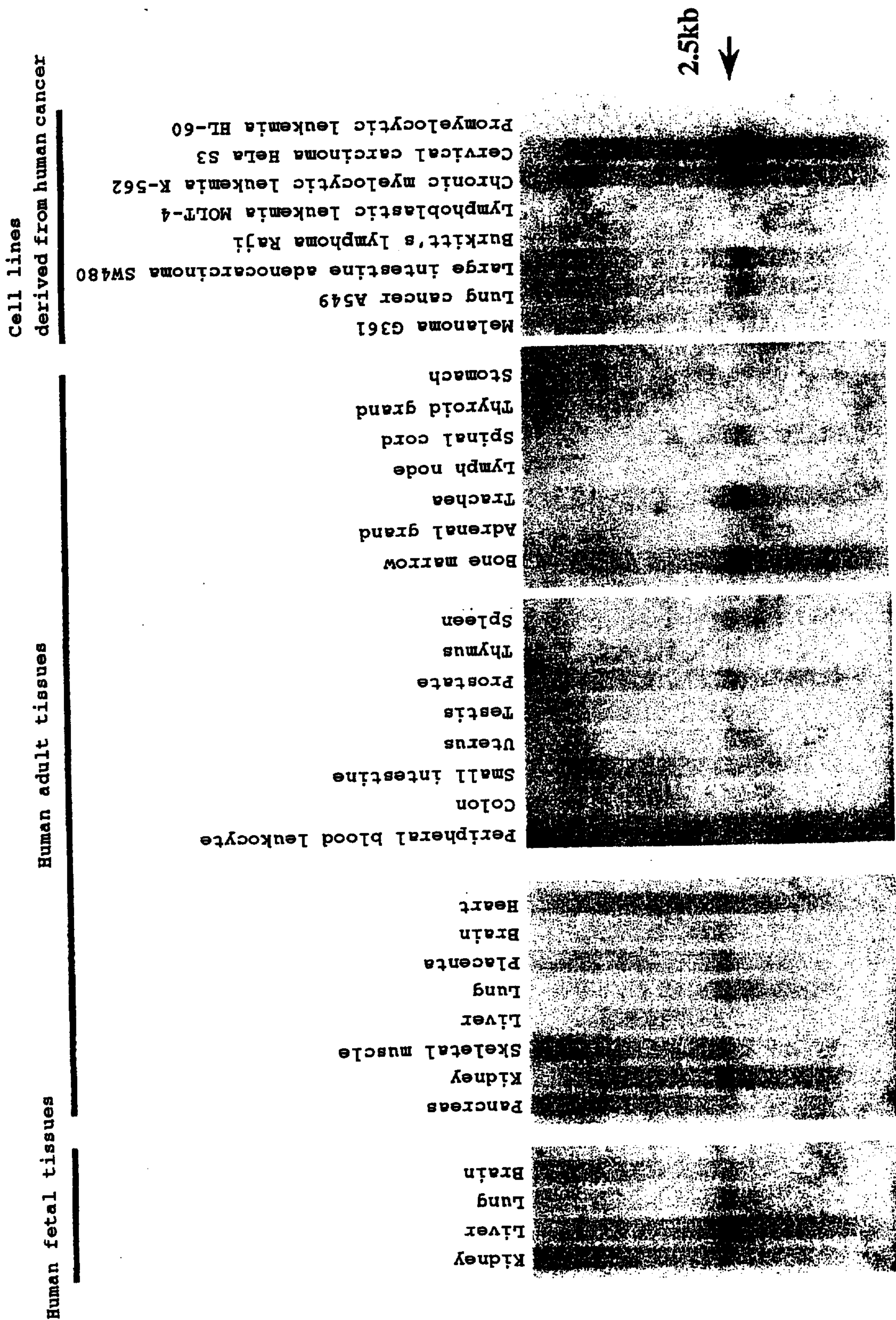
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Figure 1



2 / 1 3

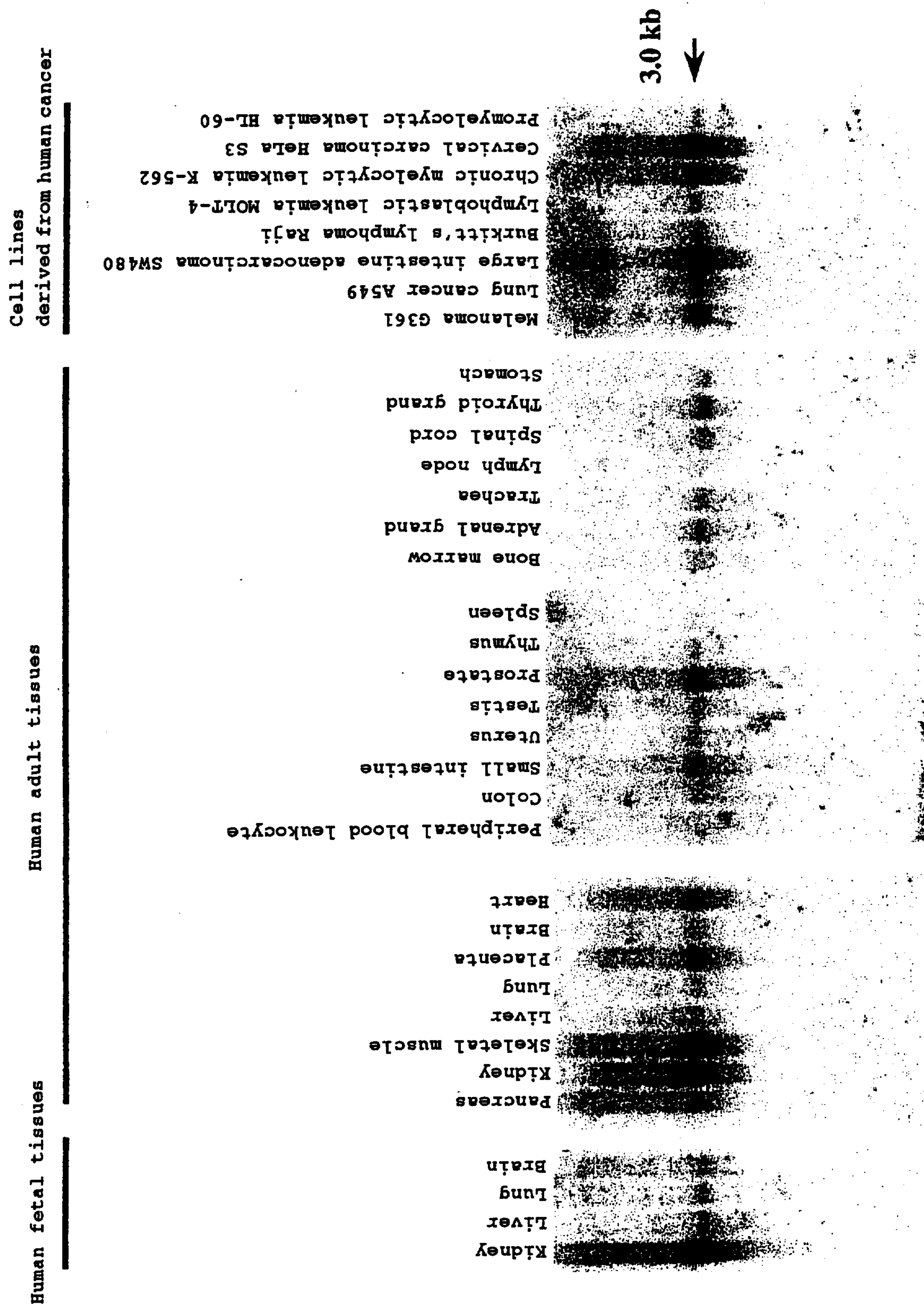
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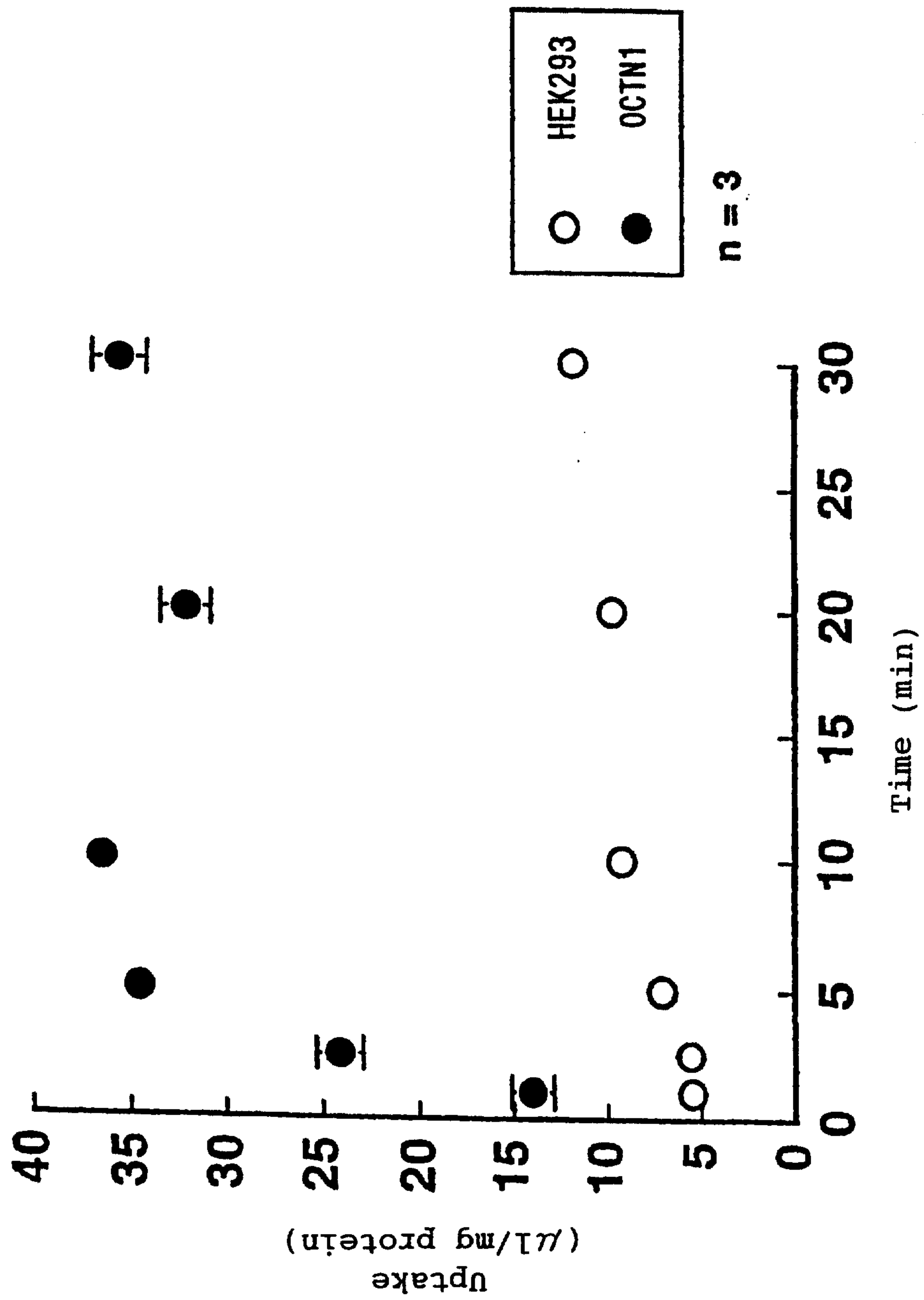
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Figure 4



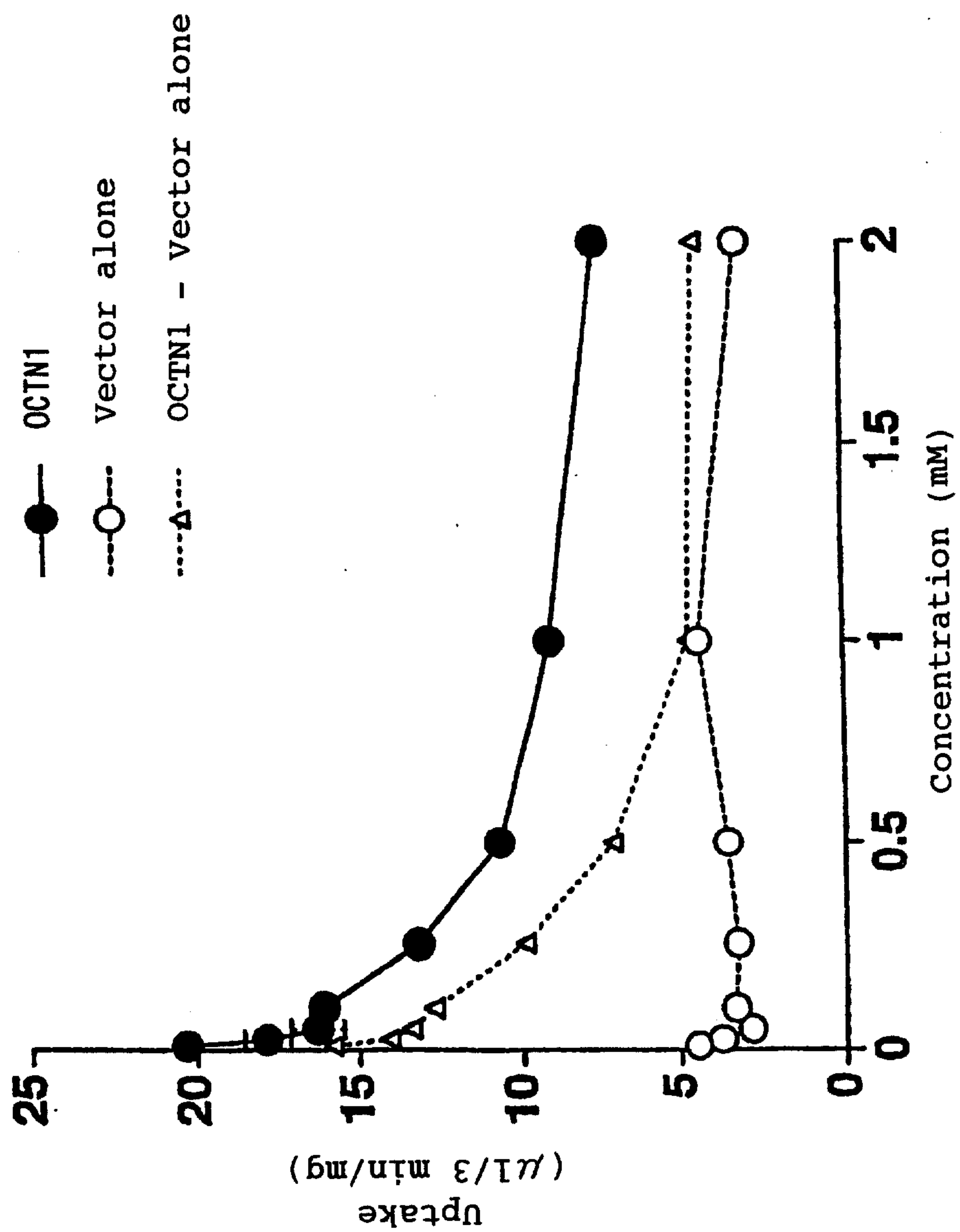
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Figure 5



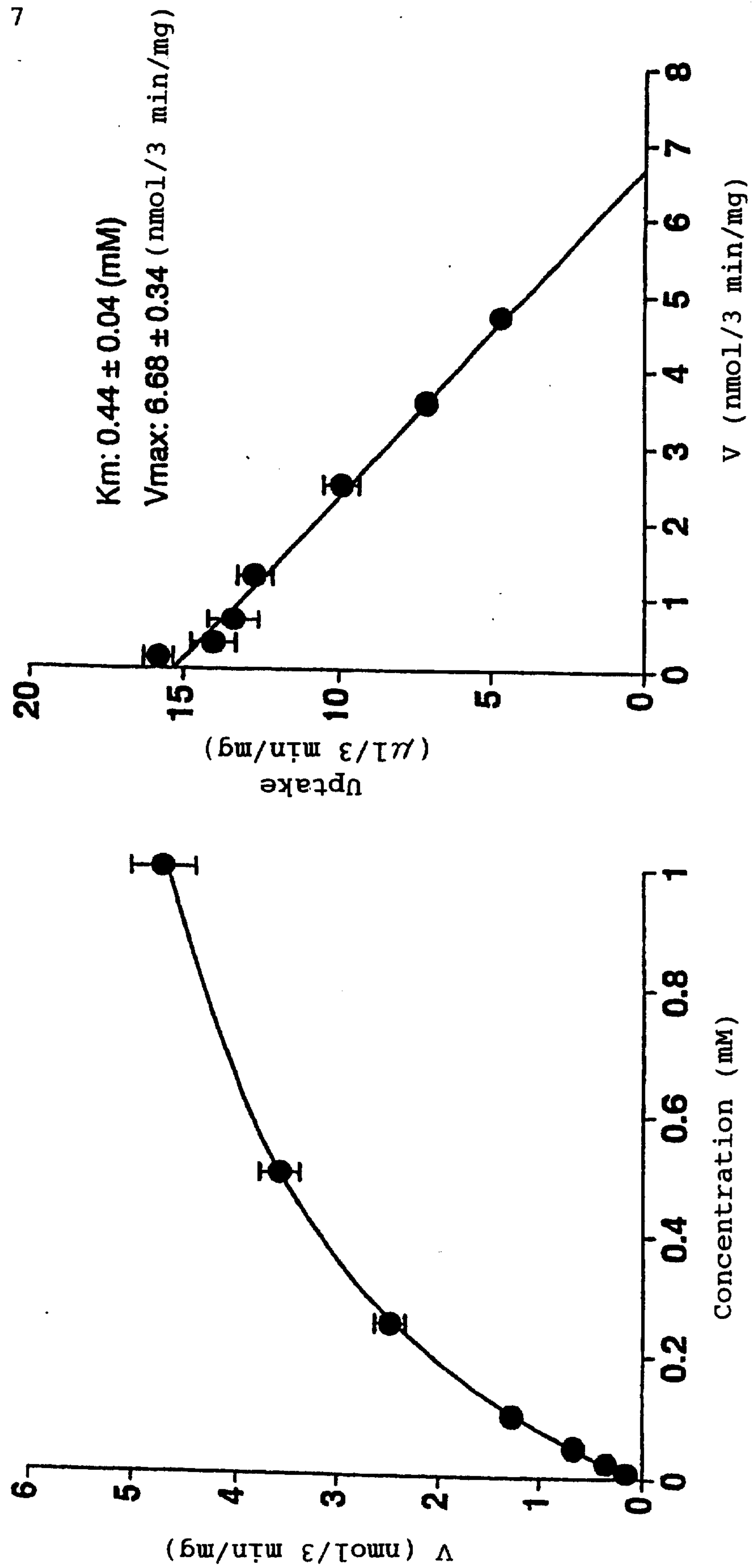
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Figure 6



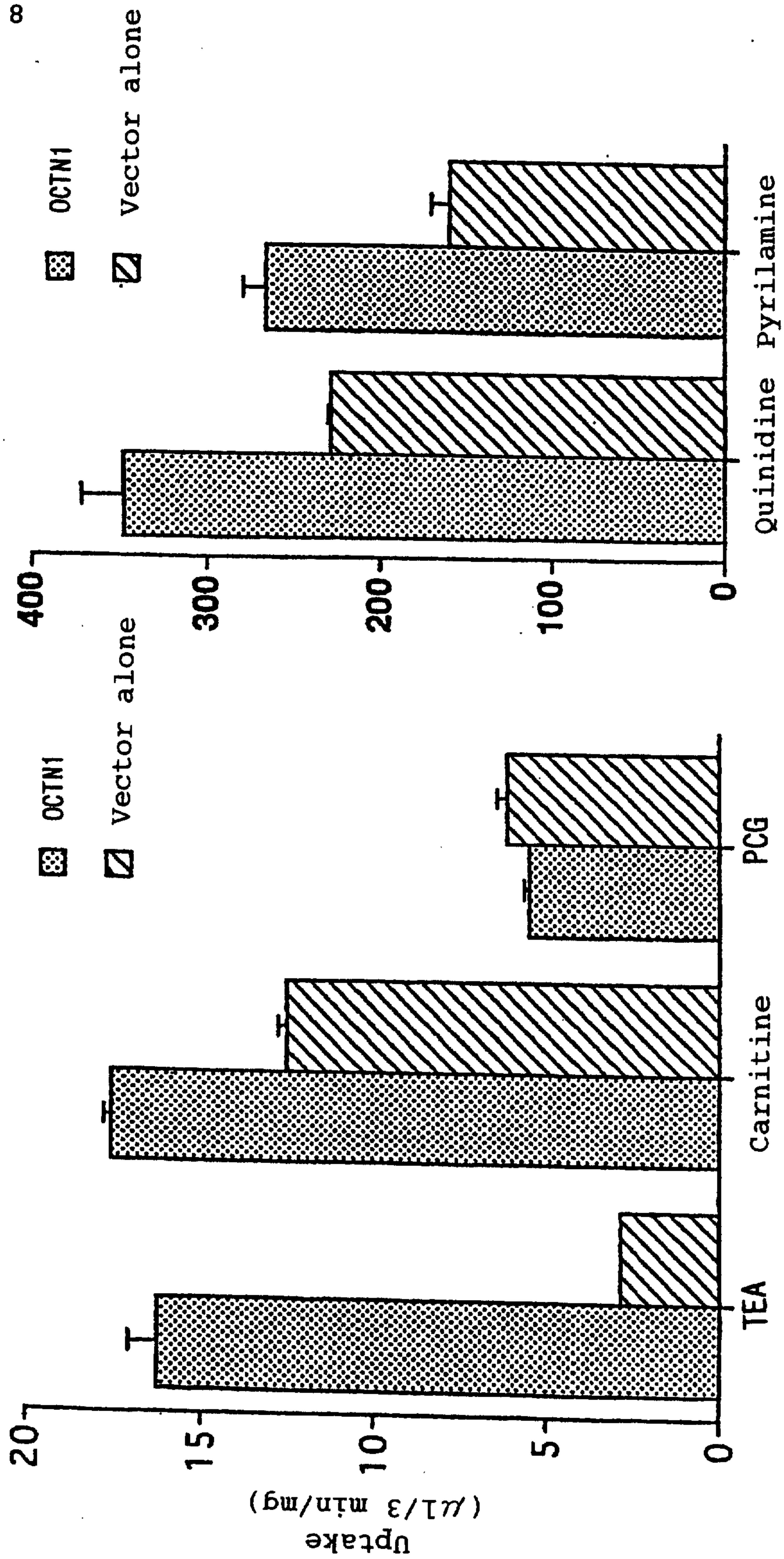
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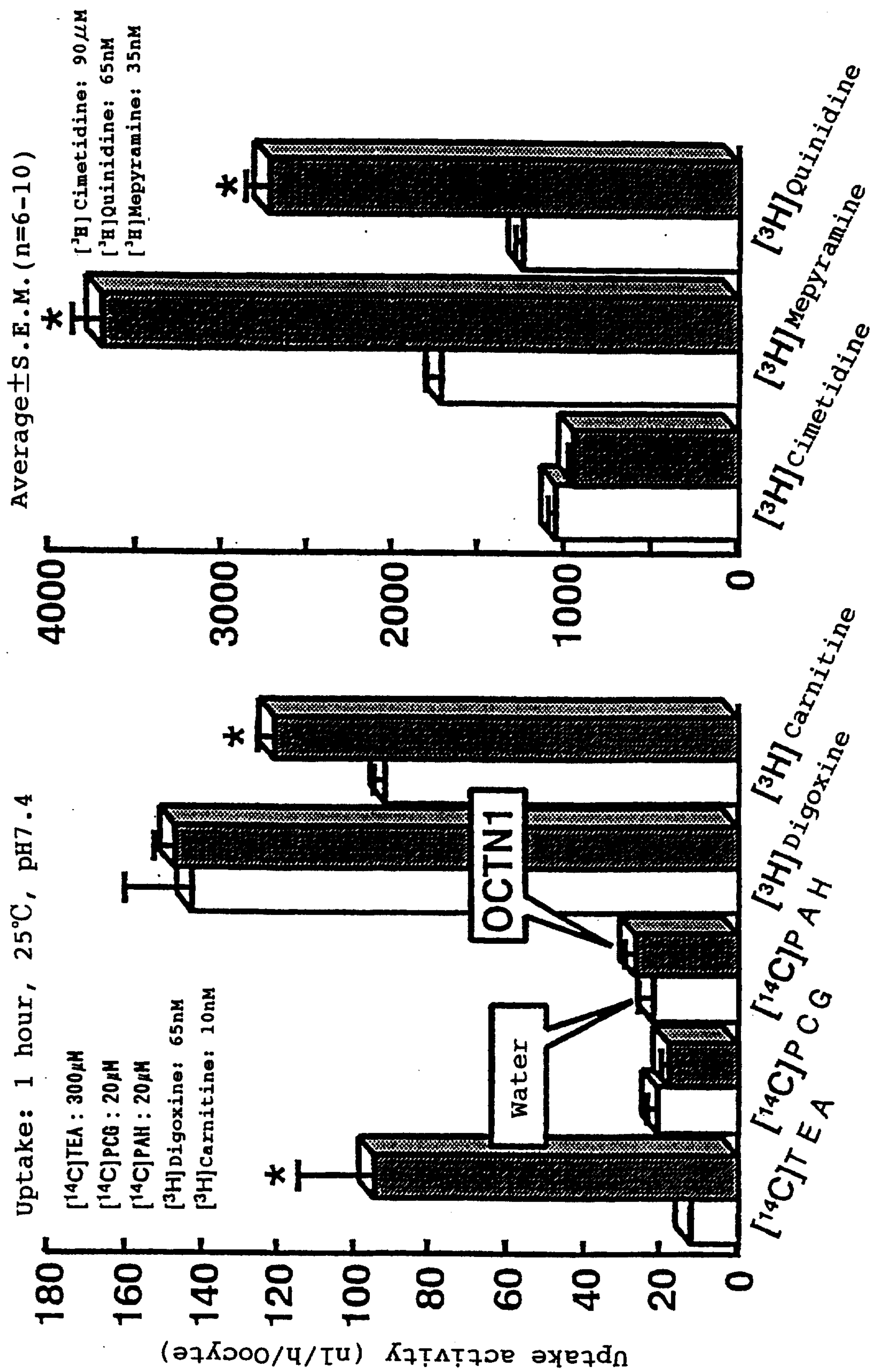
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Figure 8



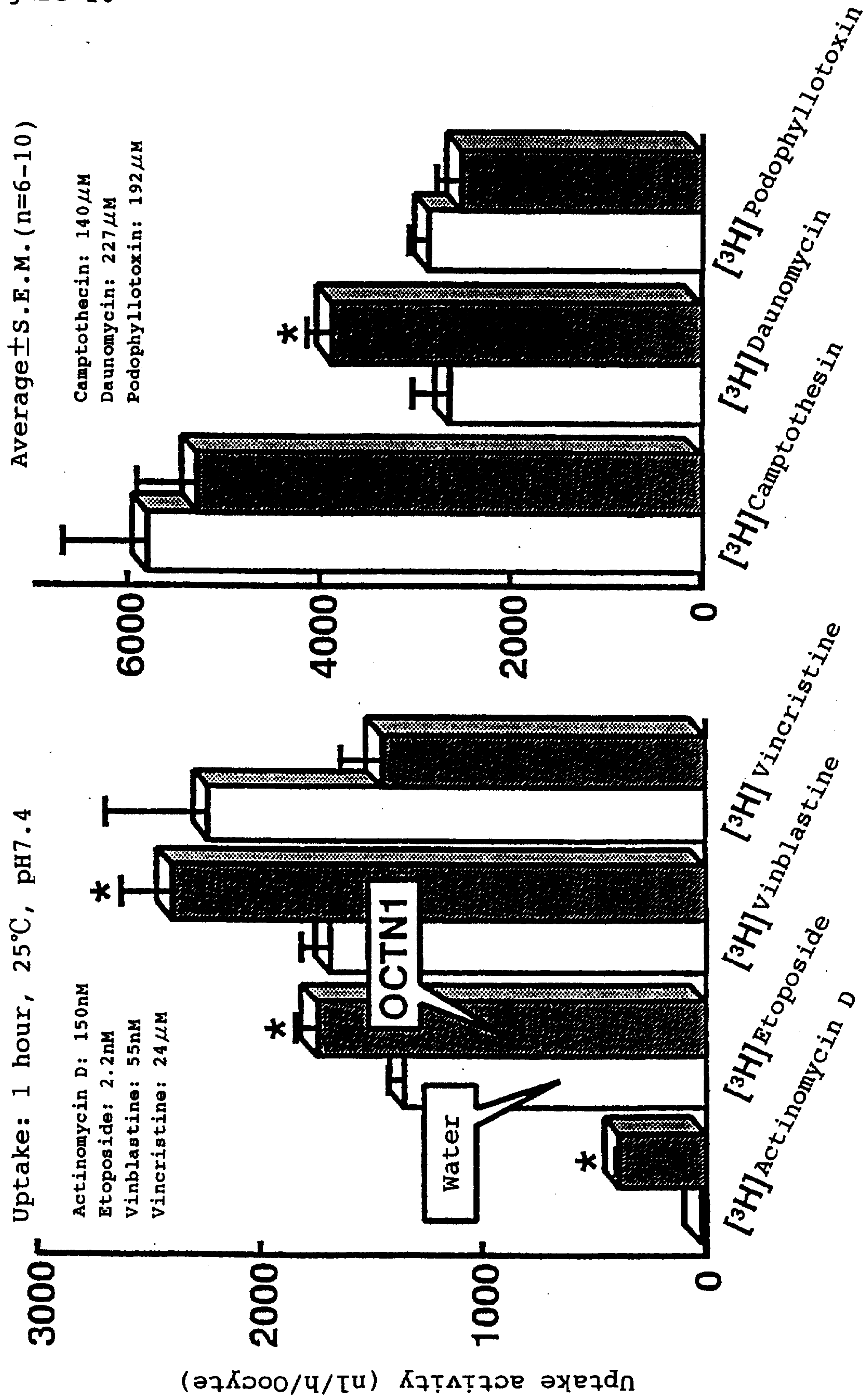
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Figure 9



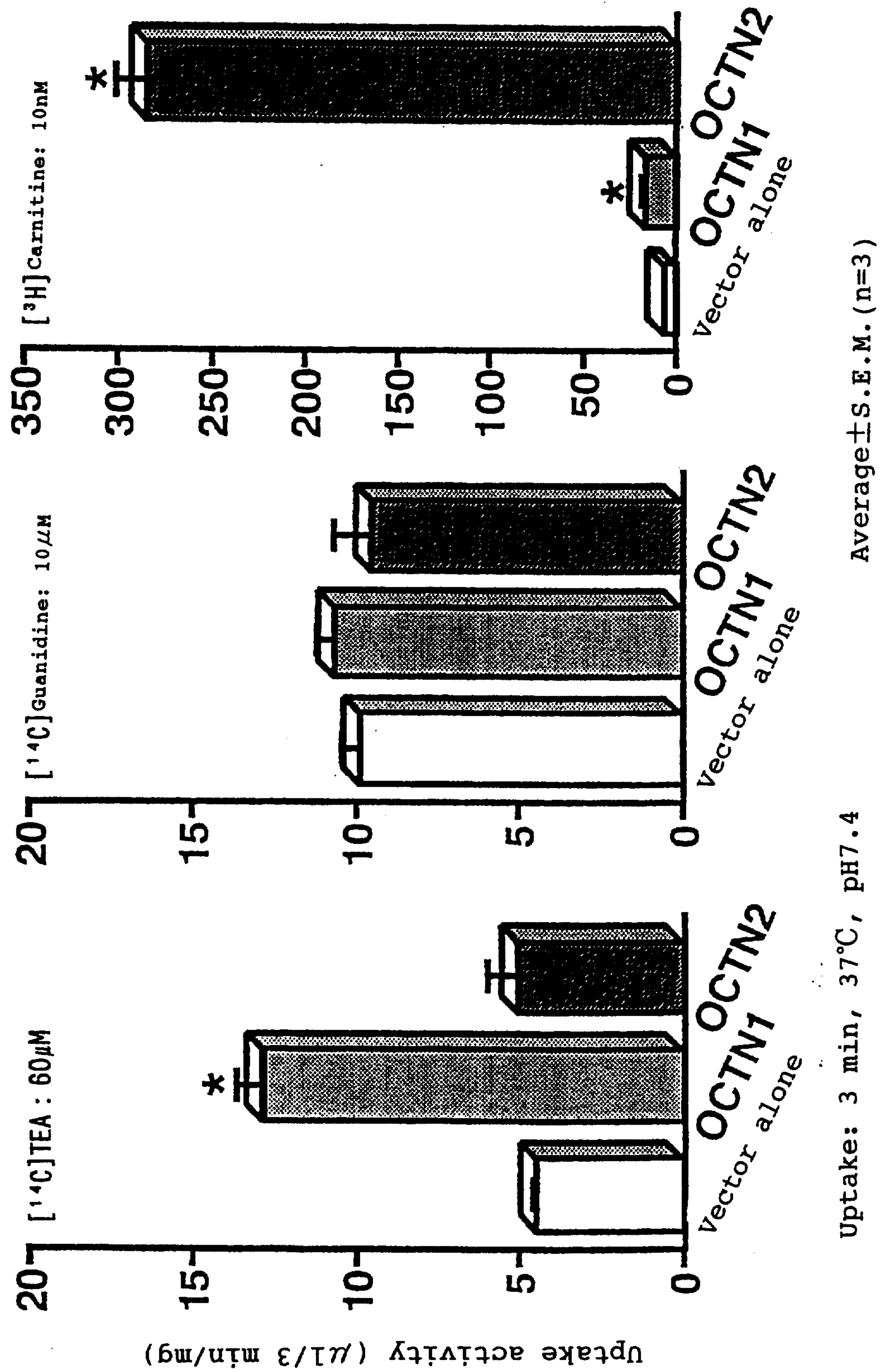
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Figure 10



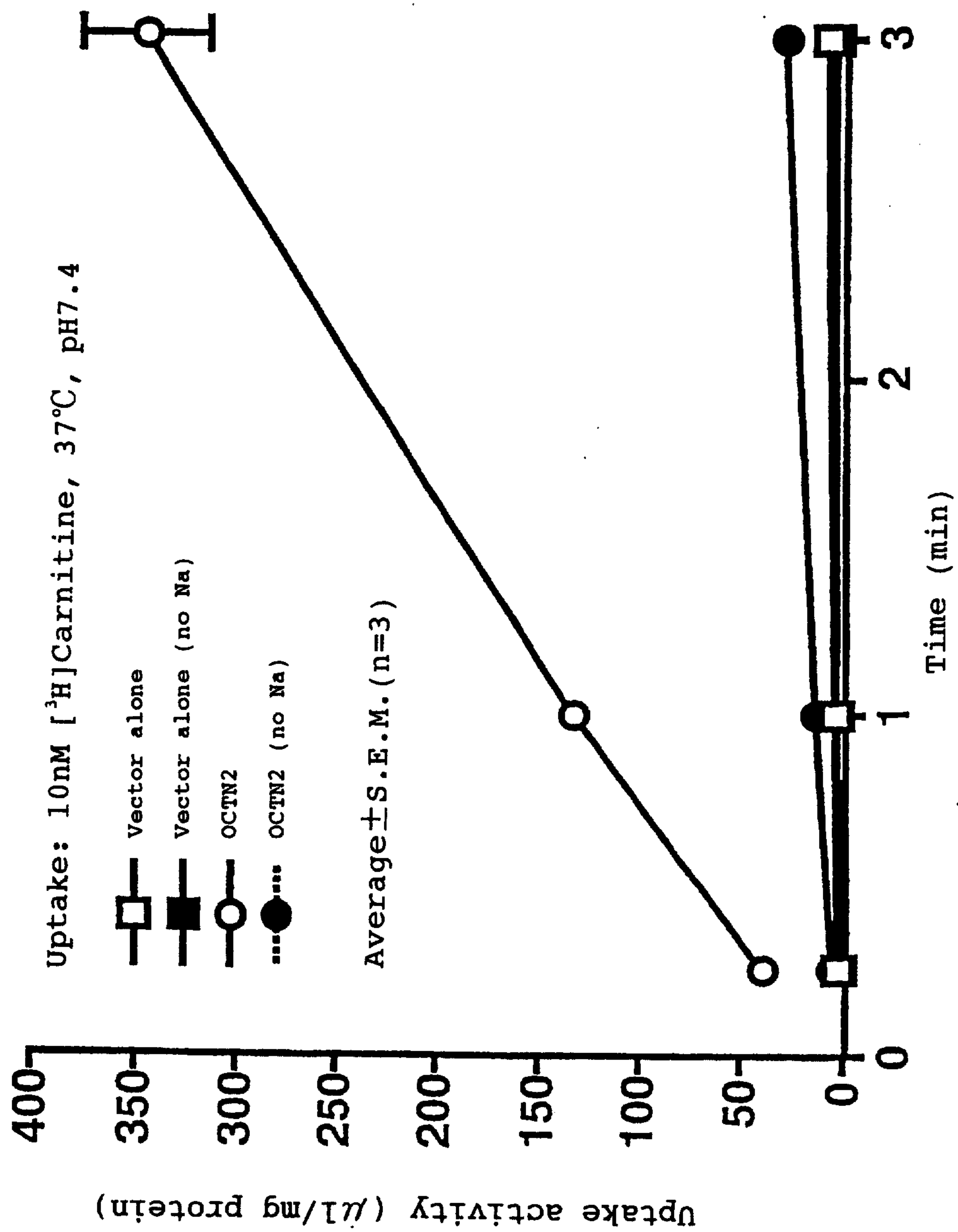
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Figure 11



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Figure 12



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Figure 13

