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(54) Title: TELLURIUM DERIVATIVES FOR PREVENTION AND TREATMENT OF NEURODEGENERATIVE PROCESSES

(57) Abrégé/Abstract:
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TELLURIUM DERIVATIVES FOR PREVENTIOIN AND TREATMENT OF NEURODEGENERATIVE PROCESSES

BACKGROUND OF THE INVENTION:

Field of the Invention

Recent evidence now indicates that the process of apoptosis primarily contributes to nerve cell death, a central feature of human neurodegenerative processes and diseases. This invention represents a novel neuroprotective modality with the use of a synthetic non-toxic tellurium compound. These compounds prevent neuronal death by interfering with apoptosis.

Description of the Related Art

Neuroprotective treatment to slow down, prevent, or even reverse degenerative processes are needed. The possibility of treating degenerative diseases with neurotrophic factors has motivated research for dopaminotrophic factors. Several neurotrophic factors have shown promise in the rescue of dopaminergic neurons in vitro such as basic fibroblast growth factor (bFGF), epithelial growth factor (EGF), insulin-like growth factor (IGF), and brain-derived neurotrophic factor (BDNF). However, their effectiveness in vivo has been for the most part somewhat less promising. Neurotrophic factors often cannot reach their target receptors since they rapidly degrade in the blood stream and cannot pass through cell membranes or the blood brain barrier. Alternatively, glial-derived neurotrophic factor (GDNF) has been found to specifically enhance the survival of midbrain dopaminergic neurons in vitro and exert a protective effect on degenerating dopaminergic neurons in vivo. Similarly, insulin-like growth factor 1 (IGF-1) has been found to prevent brain cells from dying after an asphyxial or ischemic brain insult.

Evidence now shows that some drugs can stabilize, reinforce or even regenerate neurotubules within the central or peripheral neurons of a human nervous system. Certain drugs, such as brimonidine and various beta-adrenergic blocking agents, have been accepted as neuroprotective drugs that can protect the central nervous system from acute ischemia and crush trauma in humans While certain methods and chemical compositions have been developed which aid in inhibiting, remitting, or controlling neurodegeneration, new methods and pharmacotherapeutic agents which are able to slow or stop such neurological damage are needed. There is a great need for additional compounds useful in treating a variety of neurological conditions.

Neurodegenerative processes are generally characterized by the long-lasting course

of neuronal death and the selectivity of the neuronal population or brain structure involved in the lesion. The reasons for such a specificity are largely unknown as are generally the mechanisms of the diseases. One common feature of these diseases, however, is that the neuronal death is thought to involve apoptosis, at least in part. Neuronal apoptosis is the programmed cell death mechanism. Apoptosis is required for normal development of the nervous system but also occurs in pathological states. Extensive cell death is observed after acute brain injury, including stroke and trauma, and is thought to contribute to neurodegenerative diseases such as Parkinson's disease and Alzheimer's. Cerebral infarctions such as cerebral thrombosis and embolism are triggered by ischemia of the brain due to stenosis of blood vessels, brain thrombi or brain emboli. Treatment consists of anti-edema agents such as mannitol which improve post-ischemic cerebral edema, thrombolytic agents such as alteplase or urokinase. They do not effect neuronal death or exert a neuroprotective effect. In Parkinson's disease, there is selective degeneration of dopaminergic neurons in the nigrostriatal pathway. Treatment with L-dopa does not arrest progress of the disorder in dopaminergic neurons. Pharmacotherapeutic agents are needed to prevent apoptosis or death of the dopaminergic neurons in Parkinson's disease. Similarly, in Alzheimer's disease, a neurodegenerative disease characterized by the deposition of amyloid senile plaques, neurofibrillary tangle formation and cerebrum atrophy, apoptosis is involved in the mechanism of neuronal death in dementia in these patients. Pharmacotherapeutic agents are generally held to have little efficacy in Alzheimer's dementia.

The neurotrophin family of soluble peptide factors is required for the correct development and differentiation of the nervous system. Neutrotrophins bind receptor tyrosine kinases and activate a variety of intracellular signaling molecules which are necessary for neuron survival and differentiation .(Ebadi M., Bashir R.M., Heidrick M.L. et al, 30 Neurochem Int. 347 [1997]). The identification of the specific molecules involved in vivo has attracted considerable attention. Due to the relative difficulty of studying signaling in neurons, neurotrophin signaling has been primarily studied using the pheochromocytoma PC12 cells as a model system. This cell line has proved useful for studying mechanisms of neuronal survival, differentiation, and cell death. PC12 cells

respond to NGF exposure by differentiating to resemble sympathetic neurons. Upon NGF exposure, PC12 cells cease division, extend neuritis, become electrically excitable and express neuronal markers. Withdrawal of trophic support, either by serum deprivation of proliferating neuroblast-like PC12 cells or by NGF/serum removal from neuronally differentiated cells, leads to their apoptotic death. NGF withdrawal similarly triggers death of sympathetic neurons both in vivo and in vitro. Upon neurotrophin binding two signaling cascades have been implicated thus far in the differentiation and survival of these cells activation of the ras/erk pathway (Nakamura, T., Sanokawa, R., Sasaki, Y., et al., 13 Oncogene 1111 [1996]) and P13 Kinase/Rac signaling (Raffioni, S., Bradshaw, R.A., 89 Proc. Nat'l Acad. Sci. 9121 [1992]). The ras/erk signaling pathway appears to be extremely important in mediating NGF induced differentiation of PC12 cells. Both ras and its signaling intermediates raf, mek and erk kinases are critical for this activity (Cowley, S., Patterson, H., Kemp, P. et al., 77 Cell 841 [1994]). This has been demonstrated by studies showing NGF independent differentiation of PC12 cells expressing constitutively active forms of these intermediates or inhibition of NGF-induced differentiation by expression of their dominant interfering forms. The erk pathway has been implicated in NGF-mediated PC12 cell survival (Xia, Z., Dickens, M., Raingeaud, J. et al., 270 Science 1326 [1995]) and seems required for NGF mediated cell cycle arrest. Protection of neuronal cells from death evoked by withdrawal of trophic support by agents like N-acetyl cysteine has been shown to be mediated by the activation of the ras/erk pathway and not by their antioxidative properties. In response to loss of trophic support, PC12 and other cell types show an increased JUN kinase (JNK) activity. Evidence has been provided with PC12 cells that this increase is required for death, and a model has been proposed in which survival occurs when the elevation of JNK activity is suppressed and erk kinase activity is stimulated (Id.). JNK/p38 activates the ICE proteases thereby leading to apoptotic cell death. Previous studies have shown that multiple molecules prevent the death of naïve and neuronal PC12 cells deprived of trophic support. Bc12 has been shown to protect assorted cell types from death evoked by various stimuli. In particular, this protein suppresses death of PC12 cells and sympathetic neurons induced by withdrawal of trophic support, probably via inhibition of JNK and suppression of

cytochrome c release from mitochondria followed by inhibition of caspases. It therefore follows that interference with one or more of the signaling molecules that participate in the pathways that lead to apoptotic death will confer protection from loss of trophic support or other stress conditions.

The non-toxic immunomodulator AS101 first developed by the present inventors has been shown to have beneficial effects in diverse preclinical and clinical studies. Most of its activities have been attributed in part to the stimulation of endogenous production of a variety of cytokines. AS101 decreases the Th2 cytokine IL-10 in both mice and human cells, which was followed by a simultaneous increase of specific cytokines, among which are IL-1a, IL-6, stem cell factor (CSF), IL-12, IL-6, IFNγ, and IL-2. These immunomodulating properties play a crucial role in preclinical studies demonstrating the protective effects of AS101 in parasite and viral infected mice models, in autoimmune diseases (such as Systemic Lupus Erythematosis), and in a variety of tumor models (where AS101 had a clear anti-tumoral effect). AS101 has also been shown to have protective properties against lethal and sublethal effects of irradiation and chemotherapy, including protection from hemopoietic damage and alopecia, resulting in increased survival. The protective effects of AS101 have been attributed to its ability to increase the endogenous production of IL-1α and IL-6. Phase I and II clinical trials with AS101 on cancer patients showed it was non-toxic and exerted immunomodulatory effects that are associated with its beneficial clinical effects.

The tellurium pharmaceutical compounds of the invention act directly to suppress neuronal death. Unlike heretofore used pharmacotherapeutic agents, the compounds directly prevent or otherwise control the dysfunction, degeneration or necrosis of neurons.

SUMMARY OF THE INVENTION:

The subject invention pertains to administration of an effective amount of a tellurium compound for the prevention or treatment of neurodegenerative diseases and processes. More particularly, the invention concerns novel tellurium compounds, pharmaceutical compositions containing these compounds and uses thereof.

The compounds of the invention are useful for various non-therapeutic and therapeutic

purposes. The term "neurodegenerative disorder" as used herein refers to an abnormality in a mammal in which neuronal integrity is threatened. Neuronal integrity can be threatened when neuronal cells display decreased survival or when the neurons can no longer propagate a signal. Examples of neurodegenerative processes include stroke syndromes, subarachnoid hemorrhage, brain dysfunction post-brain surgery, disorders of the nervous system due to hypoxia, hypoglycemia, brain or spinal damage, intoxication with drugs or gases, administration of chemotherapy, alcohol and the like and examples of neurodegenerative disorders include Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, myasthenia gravis, HIV-related encephalitis, cervical spondylosis, multiple sclerosis, Down's syndrome, and Huntington's chorea. A key to curing these diseases is control of neuronal death including apoptosis. The tellurium compounds of this invention may be administered systemically to one who is afflicted with neurodegenerative diseases or to patients who are believed to be susceptible to such diseases.

Accordingly, it is a primary object of the invention to provide a method for the prevention or treatment of neurodegenerative diseases which uses a tellurium based compound.

It is also an object of this invention to provide a novel composition of a neuroprotective agent such as neurotropic growth factors and a tellurium compound.

These and other objects of the invention will become apparent from a review of the specification.

BRIEF DESCRIPTION OF THE DRAWINGS:

FIGURE 1 shows activation of $p21^{ras}$ by GDP/GTP exchange. AS101 (ammonium trichloro(dioxoethylene-O,O') tellurate) was incubated with recombinant $p21^{ras}$ for 10 minutes.

FIGURE 2 shows activation of ERK1/ERK2 by AS101 using myelin basic protein as substrate. NIH3T3 cells were incubated with AS101 for 10 minutes with or without farnesyl transferase inhibitor.

FIGURE 3 shows that treatment of PC12 cells with AS101 induced neuronal differentiation in a dose-dependent manner.

FIGURE 3a shows AS101 induced neuronal differentiation in PC12 cells.

FIGURE 3b shows that treatment with AS101 of PC12 cells expressing the dominant negative form (N17) of ras did not induce neuronal differentiation.

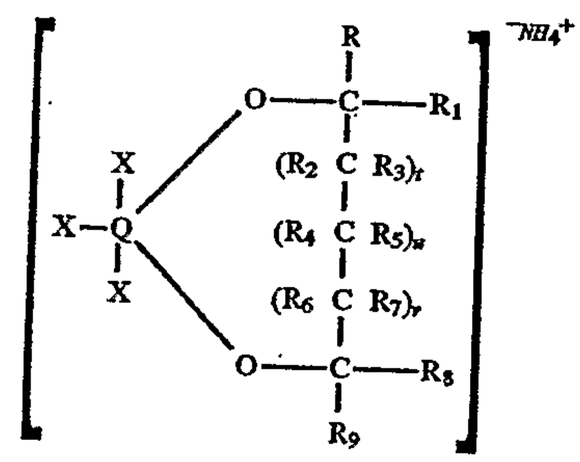
FIGURE 3c shows that treatment of with AS101 of PC12 cells expressing a point mutation in CSY118 of P21ras did not result in neuronal differentiation.

FIGURE 4 shows that in cells incubated with AS101 for 15 minutes, AS101 can activate p21ras downstream effector molecules c-raf-1.

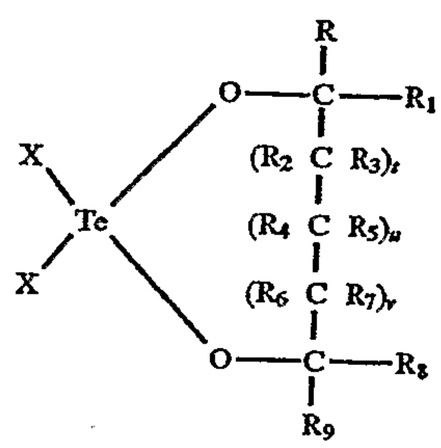
FIGURE 5 shows that in cells incubated with AS101 for 24 hours, AS101 results in a pronounced increase in p21waf protein expression in a dose dependent manner.

DETAILED DESCRIPTION OF THE INVENTION:

The tellurium compounds for use in the invention include those of the formula:



or



or

TeO_2 or complexes of TeO_2 (C)

or

$PhTeCl_3$ (D)

or

TeX_4, when X is Cl, Br or F

or the following complex: $TeO_2.HOCH_2CH_2OH.NH_4Cl$;

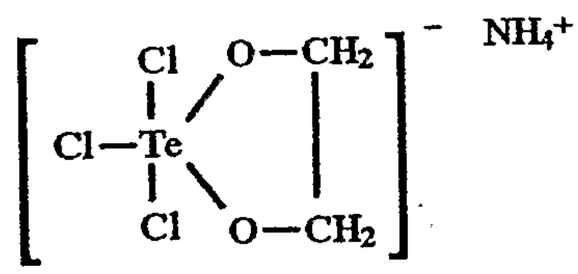
or

$(C_6H_5)_4P+(TeCl_3(O_2C_2H_4))-$ (E)

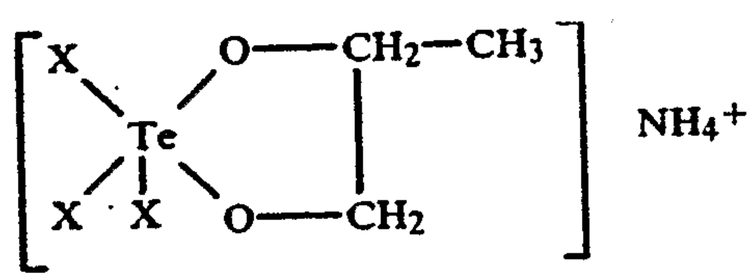
wherein t is 1 or 0; u is 1 or 0; v is 1 or 0; R, R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, and R_9 are the same or different and are independently selected from the group consisting of hydrogen, hydroxyalkyl of 1 to 5 carbons, hydroxy, alkyl or from 1 to 5 carbon atoms, halogen, haloalkyl of 1 to 5 carbon atoms, carboxy, alkylcarbonylalkyl of 2 to 10 carbons, alkanoyloxy of 1 to 5 carbon atoms, carboxyalkyl of 1 to 5 carbons atoms, acyl, amido, cyano, amidoalkyl of 1 to 5 carbons, N-monoalkylamidoalkyl of 2 to 10 carbons, N,N-dialkylamidoalkyl of 4 to 10 carbons, cyanoalkyl of 1 to 5 carbons alkoxy of 1 to 5 carbon atoms, alkoxyalkyl of 2 to 10 carbon atoms and $-COR_{10}$ wherein R_{10} is alkyl of 1 to 5 carbons; and X is halogen; while the ammonium salt is illustrated, it is understood that other pharmaceutically acceptable salts such as K+ are within the scope of the invention. The compounds with the five membered rings are preferred.

As used herein and in the appended claims, the term alkyl of 1 to 5 carbon atoms includes straight and branched chain alkyl groups such as methyl; ethyl; n-propyl; n-butyl, and the like; the term hydroxyalkyl of 1 to 5 carbon atoms includes hydroxymethyl; hydroxyethyl; hydroxy-n-butyl; the term halkoakyl of 1 to 5 carbon atoms includes chloromethyl; 2-iodoethyl; 4-bromo-n-butyl; iodoethyl; 4-bromo-n-pentyl and the like; the

term alkanoyloxy of 1 to 5 carbon atoms includes acetyl, propionyl, butanoyl and the like; the term carboxyalkyl includes carboxymethyl, carboxyethyl, ethylenecarboxy and the like; the term alkylcarbonylalkyl includes methanoylmethyl, ethanoylethyl and the like; the term amidoalkyl includes $-CH_2CONH_2$; $-CH_2CH_2CONH_2$; $-CH_2CH_2CH_2CONH_2$ and the like; the term cyanoalkyl includes
$-CH_2CN$; $-CH_2CH_2CN$; $-CH_2CH_2CH_2CN$ and the like; the alkoxy, of 1 to 5 carbon atoms includes methoxy, ethoxy, n-propoxy, n-pentoxy and the like; the terms halo and halogen are used to signify chloro, bromo, iodo and fluoro; the term acyl includes $R_{16}CO$ wherein R_{16} is H or alkyl of 1 to 5 carbons such as methanoyl, ethanoyl and the like; the term aryl includes phenyl, alkylphenyl and naphthyl; the term N-monoalkylamidoalkyl includes $-CH_2CH_2CONHCH_3$,
$-CH-_2CONHCH_2CH_3$; the term N,N-dialkylamidoalkyl includes
$-CH_2CON(CH_3)_2$; $CH_2CH_2CON(CH_2-CH_3)_2$. The tellurium based compounds that are preferred include those of the formula:



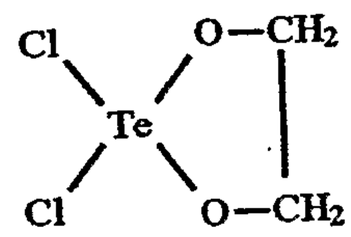
and



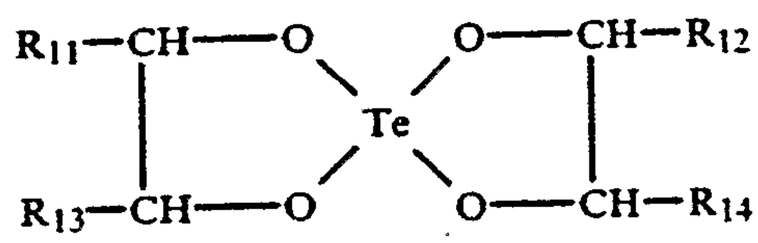
wherein X is halogen. The preferred halogen species is chloro.

Other compounds which are based on tellurium and may be used in the

practice of the invention include $PhTeCl_3$, TeO_2 and TeX_4 $(C_6H_5)_4$ P+ $(TeCl_3(O_2C_2H_4))$- (Z. Naturforsh, 36, 307-312 (1981). Compounds of the following structure are also included:



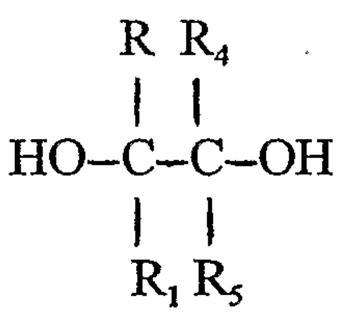
Other compounds useful for the practice of invention include:



wherein R_{11}, R_{12}, R_{13} and R_{14} are independently selected from the group consisting of hydrogen, hydroxy-alkyl of 1-5 carbons atoms, hydroxy and alkyl of 1-5 carbons atoms.

Useful dihydroxy compounds for use in the preparation of compounds of structure A or B, include those of formula I wherein R, R_1, R_4 and R_5 are as shown in the Table:

TABLE



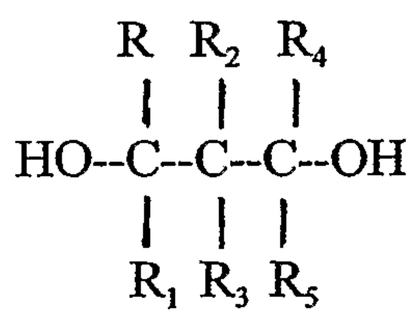
(I)

R	R_1	R_4	R_5
H	H	H	H
H	Cl	H	H
H	OCH_3	H	H
H	$COOCH_3$	H	H
H	H	CN	H
H	CHO	H	H
H	H	COOH	H
H	CH_2COOH	H	H

H	H	CH_2COOCH_3	H
H	I	H	H
H	H	Br	H
H	H	$CONH_2$	H
H	H	CH_2OH	H
H	COOH	H	H

Other dihydroxy compounds for use in the preparation of compounds A and B include those of formula II wherein R, R_1, R_2, R_3, R_4 and R_5 are as shown in the Table:

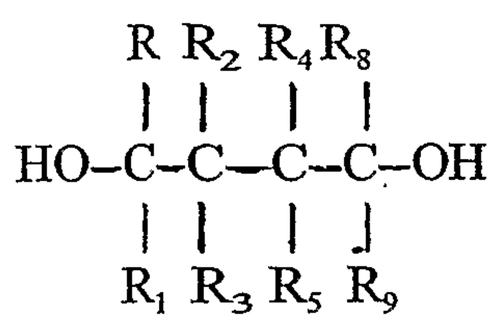
(II)



R	R_1	R_2	R_3	R_4	R_5
H	H	H	H	H	H
H	H	Cl	H	H	H
H	CH_2OH	H	H	H	H
H	H	OH	H	H	H
H	H	H	CH_3	H	H
H	H	H	CH_2Cl	H	H

H	H	H	CH_2COOH	H	H
H	H	H	CHO	H	H
H	H	H	H	H	CH_2CHO
H	H	$CONH_2$	H	H_2	CH_3
H	H	H	CN	H	H
H	H	H	H	CH_2COHN_2	H
H	H	H	$COOCH_3$	H_3	H
H	H_3	OCH_3	H	H	H

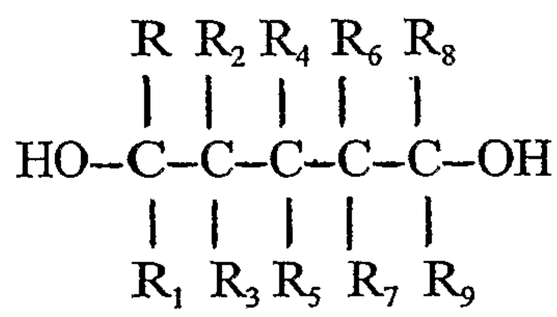
Other dihydroxy compounds for use in making compound of formula A and B include those of formula III wherein R, R_1, R_2, R_3, R_4 and R_5 are as shown in the Table.



(III)

R	R_1	R_2	R_3	R_4	R_5	R_8	R_9
H	H	H	H	H	H	H	H
H	H	Cl	H	H	H	H	H
H	H	H	H	Br	H	H	H
H	H	OCH_3	H	H	H	H	H
H	H	$CONH_2$	H	H	H	H	H
H	Br	H	H	H	H	H	H
H	H	H	H	CH_2COOH	H	H	H
H	H	Cl	Cl	H	H	H	H
H	CH_2COOH	H	H	H	H	H	H
H	H	CH_3	H	H	H	H	H
H	CH_3	H	H	H	H	H	H
H	CH_2Cl	H	H	H	H	H	H
H	H	H	I	H	H	H	H
H	CH_2CN	H	H	H	H	H	H
H	H	H	H	CH_2CH_2OH	H	H	H

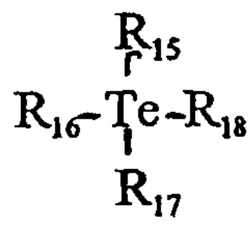
Additional dihydroxy compounds include those of formula IV wherein R, R_1, R_2, R_3, R_4 and R_5 are as shown in the Table.



(IV)

R	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
H	H	H	H	H	H	H	H	H	H
H	H	H	H	H	H	H	H	H	H
H	H	Cl	H	H	H	Cl	H	H	H
H	H	Cl	Cl	H	H	H	H	H	H
H	H	$CONCH_3$	H	H	H	Br	H	H	H
H	H	Br	H	H	H	$CON(CH_3)_2$	H	H	H
H	H	H	OCH_3	H	H	H	H	H	H
H	H	H	H	OCH_3	H	H	H	H	H
H	H	H	H	CH_2COOH	H	H	H	H	H
H	H	COOH	H	H	H	H	H	H	
H	CH_3	H	H	H	H	H	H	H	
CH_3	H	H	H	H	CH_3	H	H	H	
H	CH_2CH_3	H	H	H	H	H	Cl	H	H
H	CH_2CN	H	H	CH_2OH	H	H	H	H	H
H	H	H	I	H	H	H	H	CN	H
H	CH_2CH_2COOH	H	H	H	H	H	H	H	
H	H	CHO	H	H	H	H	H	H	
H	H	H	F	H	H	H	H	H	

Compounds of the following formula are also included:



herein R_{15}, R_{16}, R_{17} and R_{18} are independently selected from halogen, alkyl of 1-5 carbons; aryl, acyl of 1-5 carbon hydroxyalkyl of 1-5 carbons and aminoalkyl of 1-5 carbons may be made by reacting the appropriate di, tri or tetrahalotelluride with the appropriate hydroxy compound which may be of the formula: HO-R_{19};
wherein R_{19}; is alkyl of 1 to 5 carbons, haloalkyl of 1 to 5 carbons, aryl, alkylaryl, alkylamido of 1 to 5 carbons, alkylcarbonyl of 1 to 5 carbons, cyanoalkyl of 1 to 5 carbons, cyanoalkyl of 1 to 5 carbons, and an alkoxyalkyl of 2 to 10 carbons. Specific examples of R_{16} include methyl, ethyl, n-propyl, phenyl, tolyl, amidoethyl, cyanomethyl, methyloxymethyl and CH_2CH_2COOH.

These compounds are described in United States Patent No. 4,761,490.

In addition, $TeCl_4$; $TeBr_4$ and compounds which give in aqueous solution TeO_2 preferably in the form of a complex such as for example TeO_2 complex with citric acid or ethylene glycol.

The preferred compound is ammonium trichloro (dioxoethylene-O,O') tellurate.

METHODS:To assess the neuroprotective effects of AS101,PC12 cells are maintained in Dulbecco's modified Eagle's medium supplemented with 8 % heat inactivated horse serum, 8% heat inactivated fetal bovine serum, glutamine (5mM) and 50 µg/ml gentamycin at 37° C.

PC12 cells are washed in serum-free medium, resuspended to 1-5x10^6cells/ml. After 24 hours of incubation at 37°C in culture, the cells are supplemented with 3ml of medium (RPMI 1640 containing 10% FCS, 2% glutamine and 1mg/ml G418 (Life Technologies, Inc.). After another 24 hours, cells are resuspended and maintained in the selection medium. After 3-4 weeks in selective medium, transfected cells are analyzed for

via Western blotting. Results are expressed as percent p21 as compared to the negative (no drug) control.

The ras Asn-17 gene is then cloned into a mammalian expression vector. Transfection of PC12 cells with the plasmid DNA is performed with the calcium phosphate precipitation technique as described previously.

PC12 cell extracts (20μg/lane of protein) boiled under reducing conditions, are subjected to electrophoresis on 7.5 and 12.5% polyacrylamide gels and electro-transferred to nitrocellulose membranes. The membrane is blocked for one hour with 10% powdered milk in 0.2% Tween 20, Tris-buffered saline, and then incubated with the appropriate specific detecting antibodies. Immunoreactive proteins are detected with horseradish peroxidase-conjugated secondary antibodies (Amersham, Arlington Heights, IL) and a chemiluminescence reagent. For immunoprecipitation studies, immune complexes are precipitated with Protein A-Sepharose (Pharmacia) and following electrophoresis they are blotted with anti-phosphoserine or anti-phosphotyrosine antibodies.

Endogenous JNK and erk are immunoprecipitated from cell lysates with specific antibodies and their activities measured by using P^{32} ATP and glutathione s-transferase (GST) e-jun or myelin basic protein (MPB) respectively, as the substrate. Samples are run on SDS-polyacrylamide gel electrophoresis gels and subjected to Phosphorlmager analysis.

Activation of the Ras superfamily GTPases

The effect of AS101 on signaling pathways that are controlled by Ras superfamily GTPases is screened by parallel analysis of the activation of the and Ras family GTPases and their effectors. The primary method to study activation of different Ras superfamily GTPases is by (a) by pull down of activated Ras superfamily GTPases from cell lysates by binding of the specific recombinant purified effector GTPase binding domains to the activated GTP bound form. Subsequent to the pull down of the activated GTPases, the proteins are detected and quantified by western blotting. (b) Activation of GTPases effectors such as Raf or RAC is performed by reporter gene assays and (c) by direct kinase assays using immunoprecipitation kinase assays.

Detection of Apoptosis

The percentage of cells undergoing apoptosis is quantitatively determined using an Apoptosis Detection kit on the basis of their ability to bind annexin V and exclude iodide, and also by an in situ cell detection kit incorporating HTC labeling and TUNEL.

Cell cycle distribution

Cell cycle distribution studies are performed as previously described. Cells are trypsinyzed and suspended for 10 minutes at room temperature at 1.106/ml buffer containing 1mg/ml RNAse, 1% NP-40, 10μg/ml propidium iodide and 0.1% sodium citrate. Propidium iodide fluorescence is measured using a FACStar plus flow cytometer equipped with an air-cooled argon laser delivering 15mW of light at 488nM. The red fluorescence from 1.104 cells from each sample is collected through a 610nm bandpass filter.

Identification of the site of molecular interaction between AS101 and p21ras cysteine

P21ras will be cleaved by cyanogen bromide. This process yields three fragments each containing one cysteine residue: fragment 1 containing Cys51 (Mr 7,203); fragment 2 containing Cys60 (Mr 4,540) and fragment 3 containing Cys118 (Mr 6223). To confirm that Cys118 is the molecular target of AS101, a form of p21ras is generated identical to the wild type enzyme except that Cys118 is changed by a Ser residue (referred to as p21rasC118S). This modification only changes the sulfur atom of Cys118 to oxygen. The stimulation by AS101 of nucleotide exchange on GDP-preloaded p21rasC118S in vitro was determined.

In attempting to elucidate the cellular mechanisms of AS101's effects, we observed that the primary cellular target of AS101 is the small G-protein p21 ras. AS101 directly binds to recombinant p21 ras and activates it via GDP/GTP exchange (Fig. 1). In a cellular model of Jurkat T cells or NIH3T3 cells, AS101 activates ras and its downstream effector Erk. This was shown by the kinase assay of immunoprecipitated Erk using myelin basic protein as substrate (Fig. 2). Moreover, we recently showed the ability of

AS101 to activate the ras/raf/ere pathway in B16 melanoma cells. This property was found necessary for AS101's ability to cause G0/G1 cell cycle arrest. Based on these signaling properties, and the role of ras/erk in the survival and differentiation of PC12 cells, this cell line was utilized for studying the differentiating ability of AS101 and it potential ability to prevent apoptotic death caused by loss of trophic support. Treatment of PC12 cells with AS101 induced neuronal differentiation in a dose-dependent manner (Fig.3). The optimal doses were found to be 0.5 and 1 μg/ml. Morphological changes appeared in AS101-treated cells which included membrane ruffling, flattening of cells, enlarged cell bodies, and the formation of stable neuritis. The morphological appearance of AS101 treated cells did not differ from that of NGF treated cells. Treatment with AS101 of PC12 cells expressing the dominant negative form (N17) of ras did not result in their differentiation, thus implicating ras as a crucial signaling molecule in the differentiating ability of AS101. Moreover, treatment with AS101 of PC12 cells expressing a point mutation of Cys118 of P21ras did not result in cellular differentiation while it did not prevent this activity by NGF, suggesting Cys118 as the target of AS101 in the p21ras molecule (Fig. 3).

AS101 could activate p21ras downstream effector molecules c-raf-1 (Fig. 4). The ability of AS101 to induce neuronal differentiation of PC12 cells led us to study its effect on the expression of p21waf, known to increase following differentiation of cells by NGF. Treatment of PC12 cells with AS101 for 24h resulted in a pronounced increase in p21waf protein expressing in a dose-dependent manner. The effective concentrations of AS101 were similar to those including differentiation of PC12 cells (Fig. 5). Pretreatment of the cells with farnesyl transferase inhibitor, with geldanamycin (which pharmacologically depletes c-raf-1), or with PD98059 (a MEK inhibitor) abolished p21waf protein expression induced by AS101. These results imply that p21waf protein expression induced by AS101 is both ras, c-raf-1, and MAPK-dependent.

Based on the ability of AS101 to activate the ras/erk pathway, to upregulate p21waf all of which effects have been shown to mediate the survival of PC12 cells, we analyzed its ability to prevent apoptotic cell death of differentiated PC12 cells following withdrawal of trophic support. As shown in Table 1 treatment of PC12 cells with AS101

resulted in the induction of G1 arrest in a dose-dependent manner. Following incubation of the cells with AS101 for 24 hours, 68.1% of the cells stimulated with 0.5μg/ml AS101 accumulated in G1 as compared to 33% of untreated cells. More importantly, treatment of PC12 cells with anti-NGF abs 5 days following incubation of the cells with NGF, resulted in 50% apoptosis 24 hours later. Addition of 0.5μg/ml AS101 with anti-NGF abs significantly decreased the rate of apoptosis occurring one day later, while it did not significantly differ from that of control cells incubated without AS101, and amounted to 34.9%. The results are presented in Table 1:

TABLE 1

Cell Cycle Analysis of AS101 Treated PC12 Cells and Rescue by AS101 From Apoptosis Induced by NGF Withdrawal.

	Apoptotic	G0/G1	S	G2/M
CONTROL	6.3	33.9	44.9	21.2
AS101 0.1 μg/ml	8.8	39.7	43.9	16.4
AS101 0.5 μg/ml	5.3	68.1	4.0	27.9
AS101 1μg/ml	6.6	67.5	3.5	29
NGF	5.9	65.2	7.3	27.5
NGF+ anti NGF Ab	49.8	46.3	12.2	41.6
NGF+ anti NGF +Ab+ AS101	5.8	68	4.4	27.6
CONTROL + anti NGF Ab	5.3	34.9	42.3	22.7

The demonstration that neuronal death can be blocked by manipulation of the cell death program, regardless of the cell death signal, has raised enormous hopes for the treatment of neurodegenerative diseases in which the cell death signals are of unknown origin or have already occurred. In recent years apoptosis has been described in a variety of human neurodegenerative disorders, primarily based on the detection of neuronal nuclei

with apparent DNA cleavage in post-mortem brain tissue. Such nuclei definitive evidence in support of apoptosis are the electron microscopic findings of nuclear chromatin condensation in the substrantia nigra pars compacta (SNC) of PD brains.

Critical observations have been made by Tatton and Olanow suggesting that in neurodegenerative disorders, degenerating nerve cells may be in a pre-apoptotic state for some time before entering the end stages of apoptosis, as marked by chromatin condensation and DNA cleavage. Thus, neurodegenerative disorders might reflect accelerated apoptosis as a result of agonal events in neurons that were pre-apoptotic and committed to undergo apoptosis at a later time point. This observation provides an opportunity to interfere with the cell death process and to design a putative neuroprotective agent.

The tellurium compound may be administered in a variety of forms. These include orally, parenterally, rectally, nasally or via inhalation. The parenteral route of administration may be intravenously, subcutaneously, intramuscularly etc. The compounds may also be administered directly to where the dopaminergic neurons to be protected are located; i.e. directly to the brain or cerebrospinal fluid by cerebro-ventricular injection, by injection in to the cerebral parenchyma or through a surgically inserted shunt into the lateral cerebro ventricle of the brain. In general, the composition of the subject invention will be formulated such that an effective amount of bioactive tellurium compound is combined with a suitable carrier in order to facilitate effective administration of the composition. The oral administration may be as a solid dosage form i.e. tablet with conventional excipients such as lactose, microcrystalline cellulose and the like. It has been found that the tellurium compounds useful in the practice of the invention will hydrolyze in the presence of water. These hydrolyzed compositions are active in vivo and in vitro although the hydrolyzed compositions eventually decompose. For this reason, the compositions should be freshly prepared or administered orally in the dry form. Preferably, the compounds should be kept under anhydrous conditions until just prior to being used.

Pharmaceutically acceptable carriers or diluents may be, for example, binders, (e.g., syrup, gum Arabic, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone, etc), excipients (e.g., lactose, sucrose, corn starch, sorbitol), lubricants (e.g., magnesium stearate, talc,

polyethylene glycol, silica, etc.), disintegrants (e.g. microcrystalline cellulose, potato starch, etc.), wetting agents (e.g. sodium lauryl sulfate, etc.), and the like. These pharmaceutical preparations may be in the form of a solid preparation such as tablets, capsules, powders, etc., or in the form of a liquid preparation such as solution, suspension, emulsion, etc., when administered orally. When administered parenterally, the pharmaceutical preparations may be in the form of a suppository, an injection or an intravenous drip, a physiological salt solution, and so on.

Therapeutic application of AS101 and other tellurium compounds, can be contemplated to be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. In addition, the tellurium compound may be employed alone as the sole active agent or with one or more of the invention compounds, or in combination with a second active ingredient comprising, for example, a neuroprotective compound known in the art. Some examples include interferon, insulin-like growth factor 1 (IGF-1), or GDNF.

Dosages can be titrated to the individual patient. The dose of ammonium trichloro (dioxoethylene-O,O') tellurate or a pharmaceutically acceptable salt thereof varies depending on the administration route, ages, weights and condition of individual patients, or the severity of the disease, but in humans it may be in the range of from 1 to 10 mg/m^2, preferably in the range of from 2-4 mg/m^2, and most preferably 3 mg/m^2 administered on alternate days or daily in one or more divided doses.

The foregoing description of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed. Obvious modifications or variations are possible in light of the above teachings. All such obvious modifications and variations are intended to be within the scope of the appended claims.

CLAIMS:

1. Use of ammonium trichloro (dioxoethylene-O,O') tellurate (AS101) for the manufacture of a medicament for treating a neurodegenerative disease.

2. The use as defined in claim 1, wherein said medicament is formulated for parenteral administration or for direct administration to where dopaminergic neurons to be protected are located.

3. The use as defined in claim 1, wherein the compound is used in combination with a neurotropic growth factor.

4. The use as defined in claim 1 wherein said medicament is formulated for oral administration.

5. The use as defined in claim 1 wherein the compound is used in combination with an antispasticity agent or an anti-inflammatory agent.

6. The use of claim 1, wherein said medicament comprises said AS101 in admixture with a pharmaceutically acceptable carrier or diluent.

7. Use of ammonium trichloro (dioxoethylene-O,O') tellurate (AS101) for the manufacture of a medicament for the prophylaxis or treatment of a neurodegenerative disorder.

8. The use of claim 7, wherein said neurodegenerative disorder is selected from the group consisting of Alzheimer's disease, Parkinson's disease, multiple sclerosis, stroke syndromes, and amyotrophic lateral sclerosis.

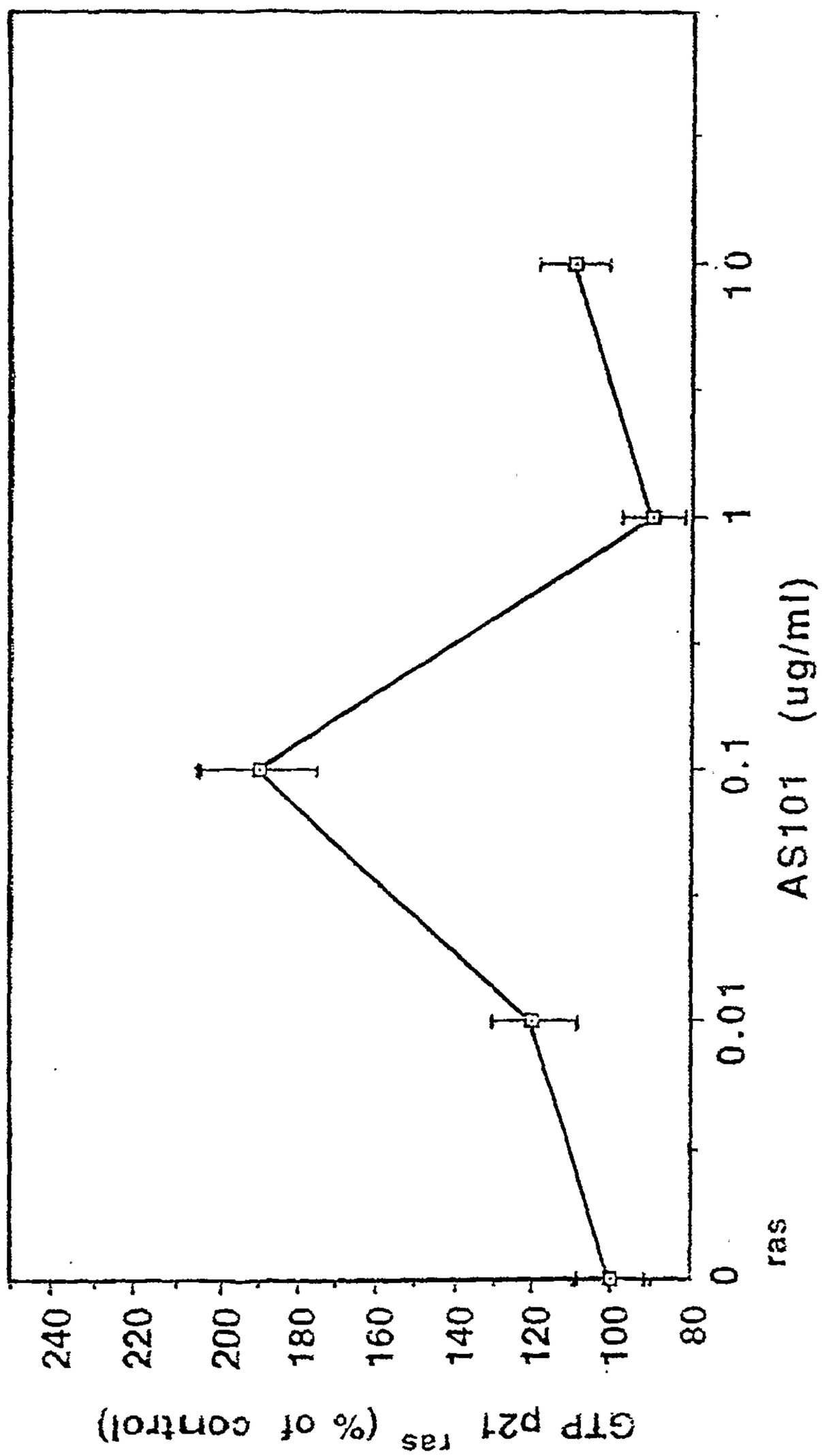


Fig. 1 Activation of p21 ras by GDP/GTP Exchange. AS101 Incubated With Recombinant p 21 ras for 10 minutes.

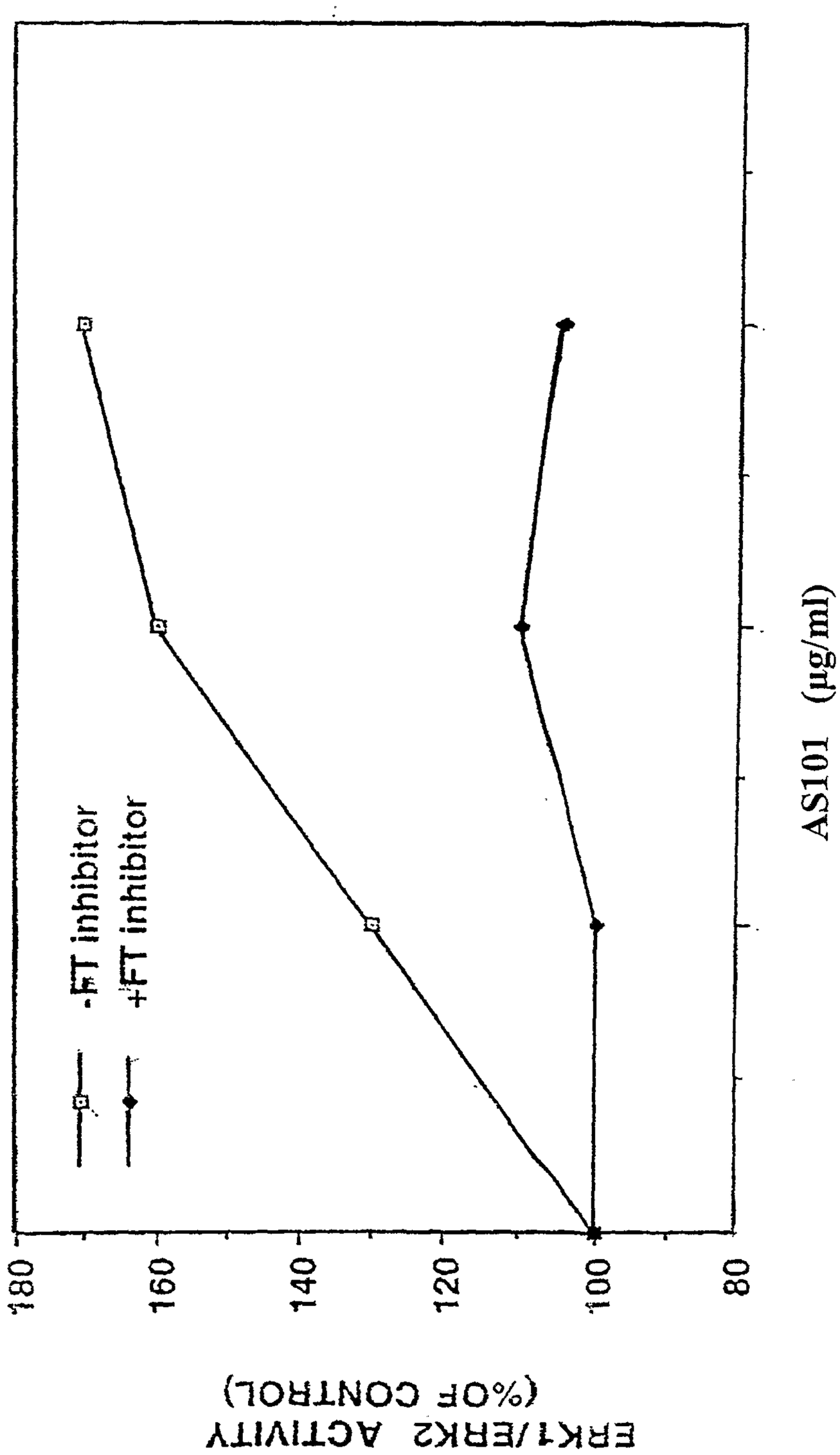
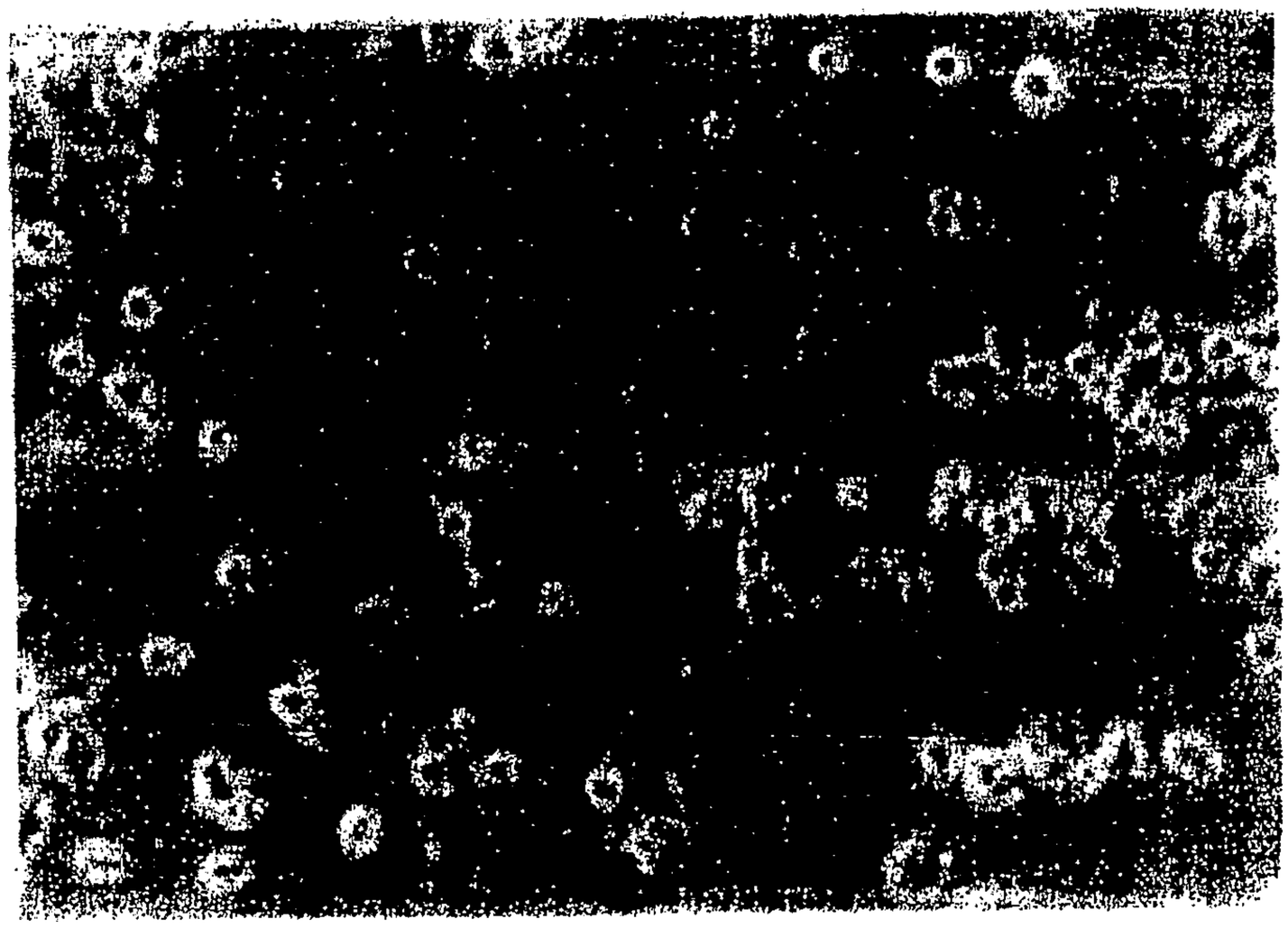


Fig. 2 Activation of ERK1/ERK2 by AS101 Using Myelin Basic Protein as Substrate. N1H3T3 Cells Incubated With AS101 for 10 Minutes With or Without Farnesyl Transferase Inhibitor.

Fig. 3 AS101 Induced Neuritis Outgrowth in PC12 Cells

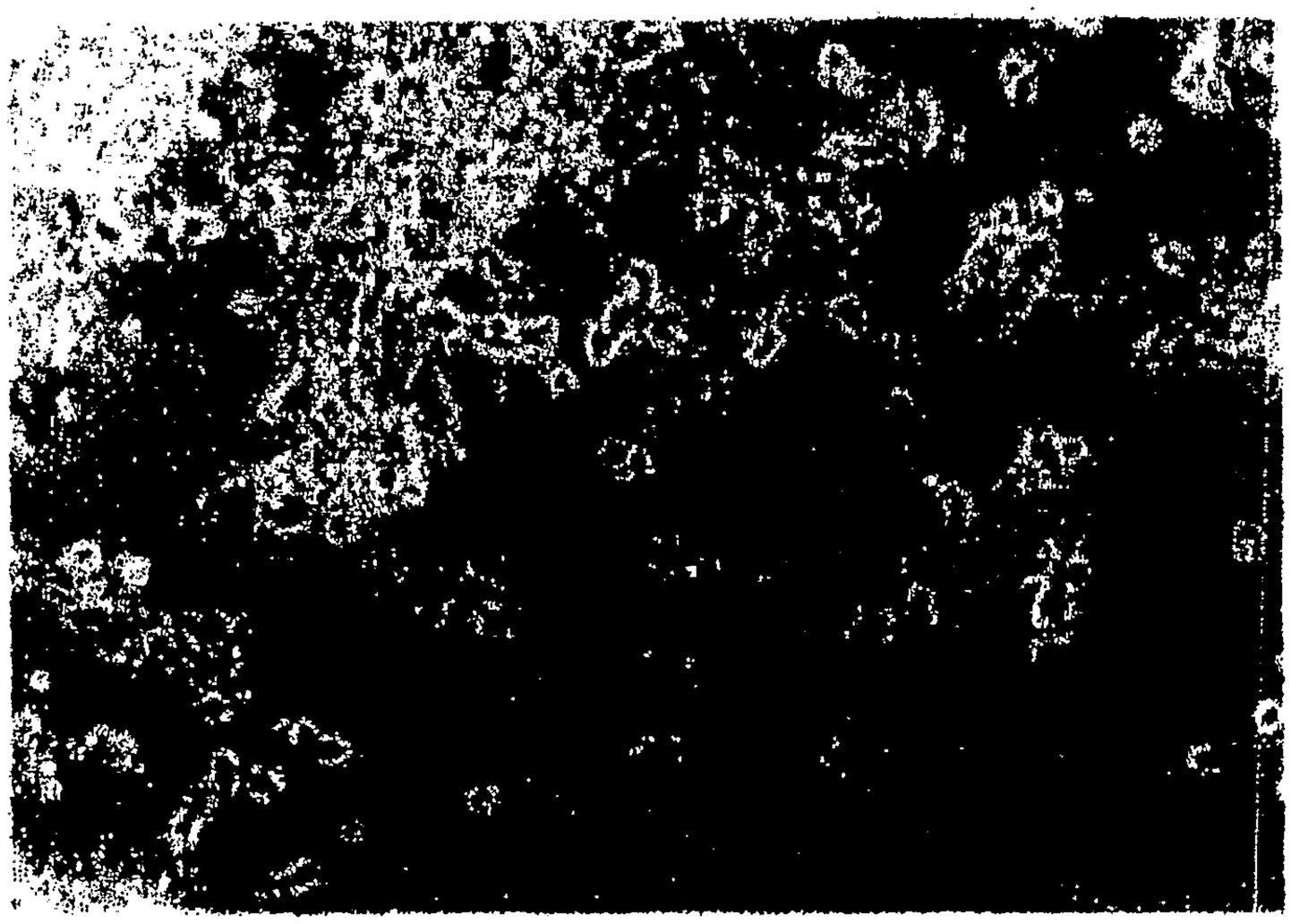
Fig. 3a PC12WT

CONTROL



AS101

(0.5 µg/ml)



NGF

(100ng/ml)

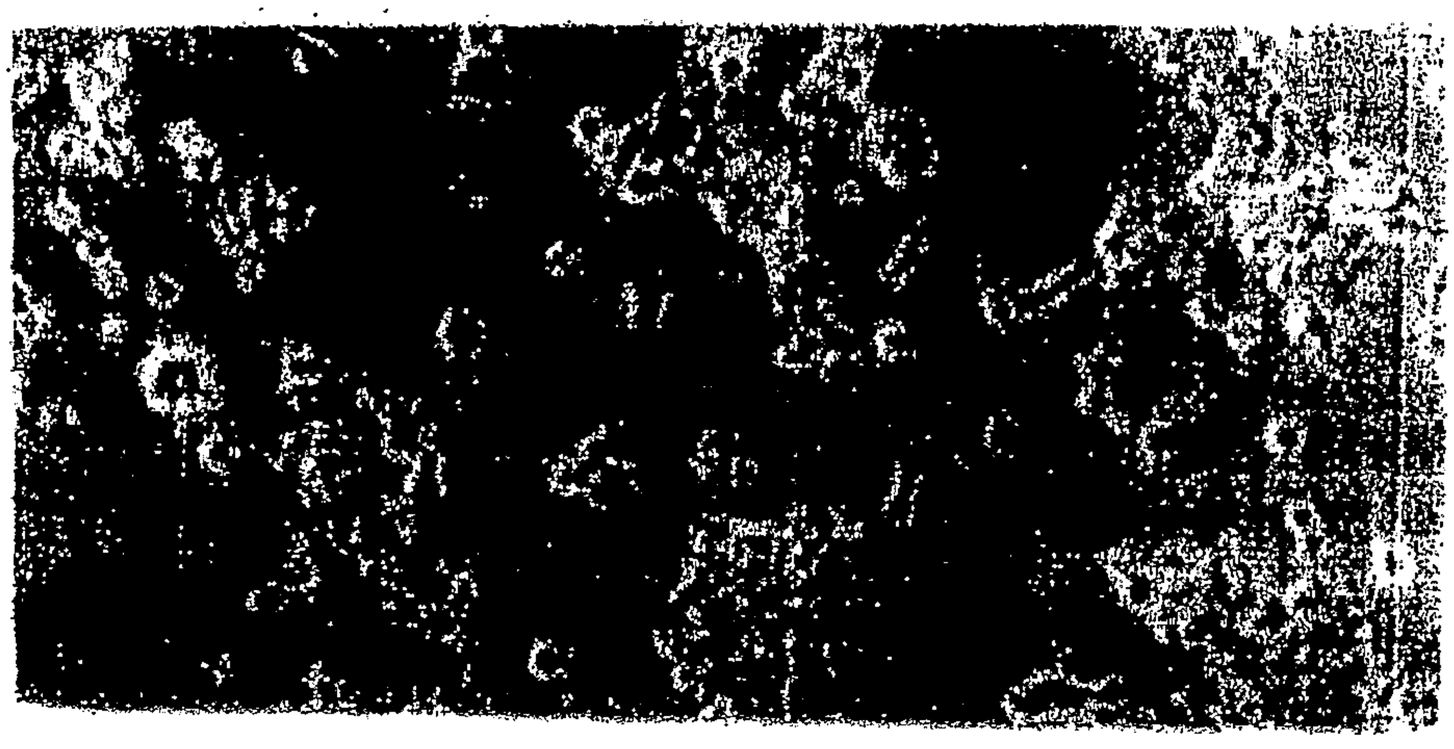
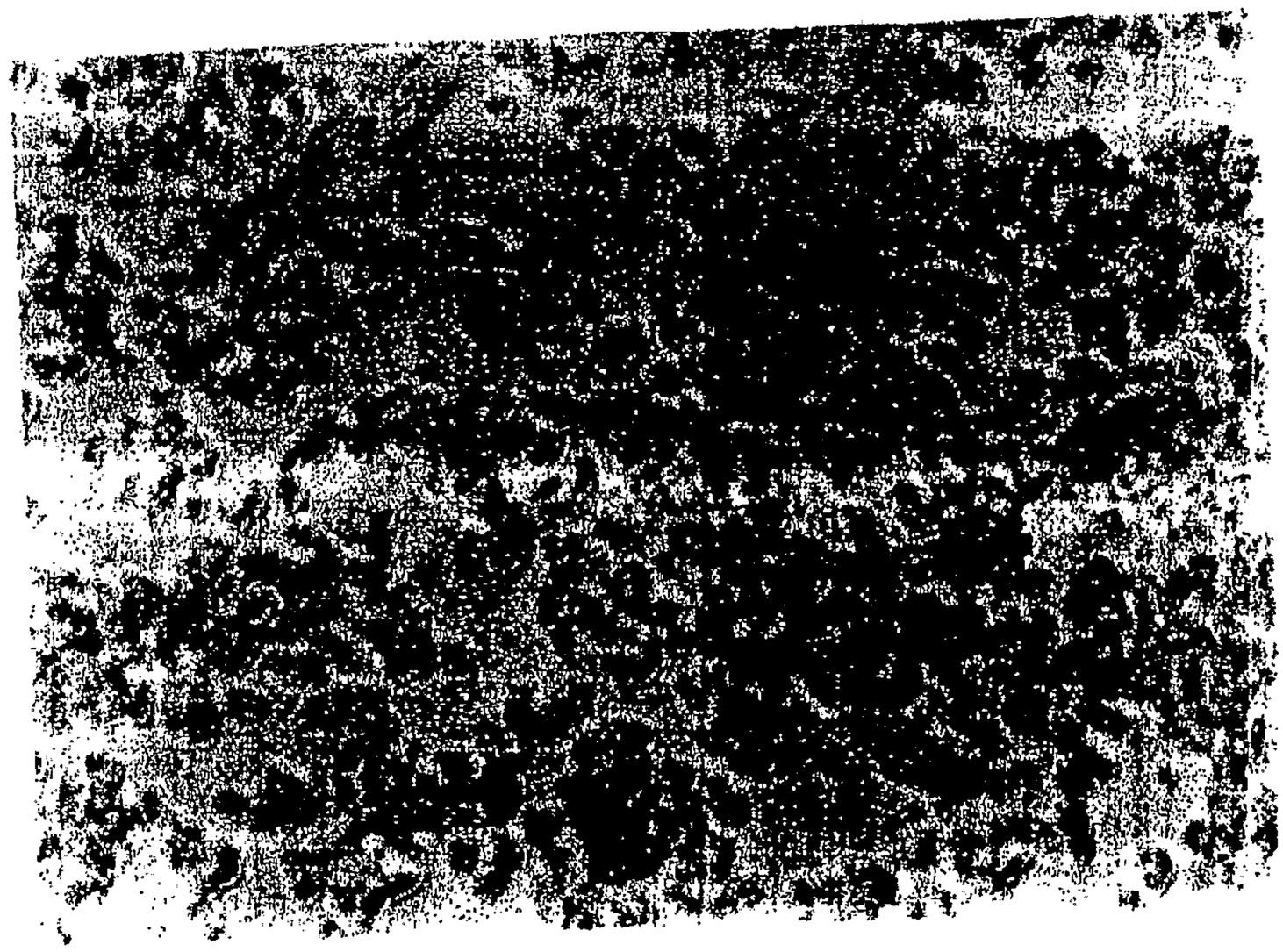


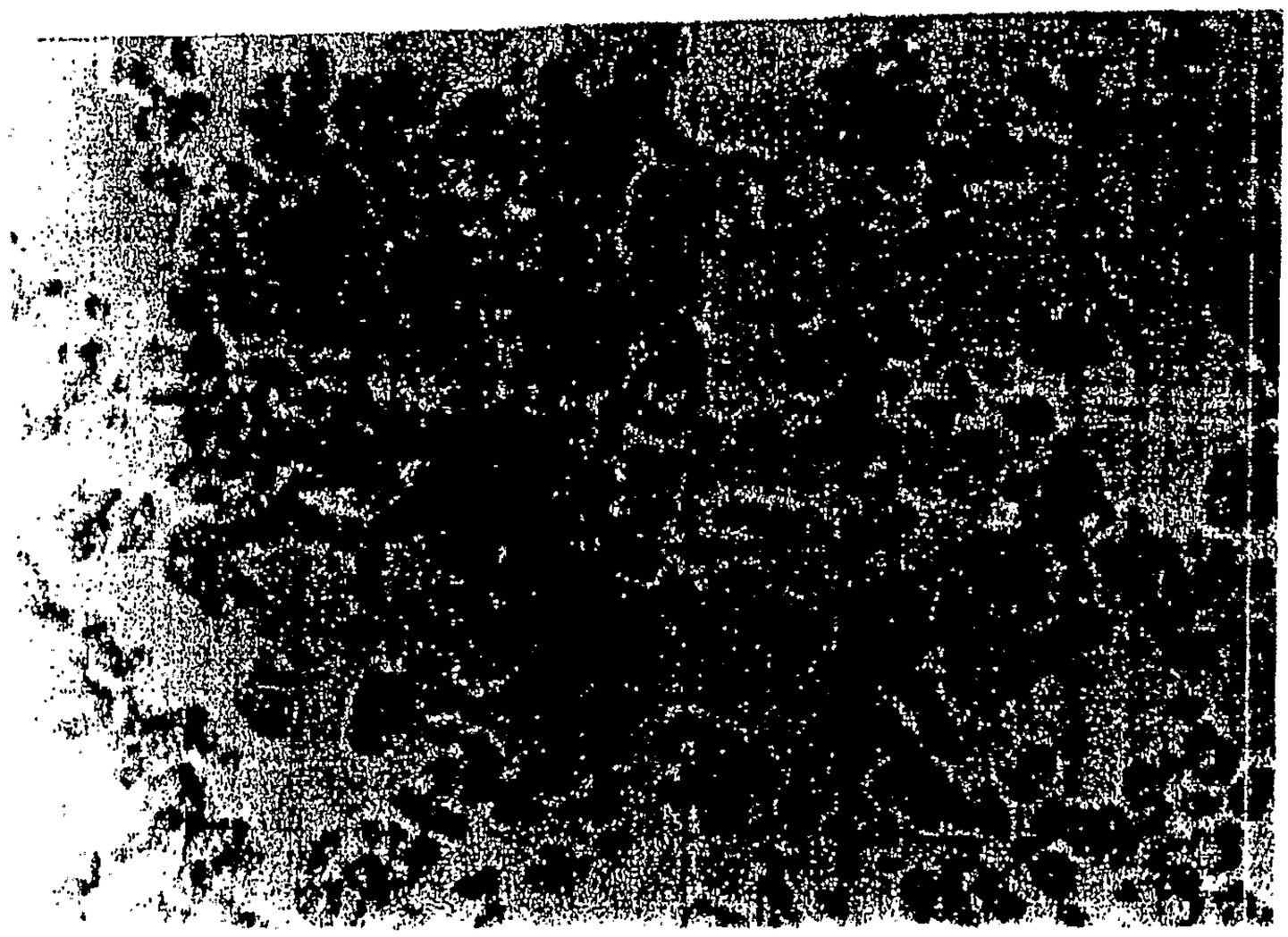
Fig. 3b N17ra5

CONTROL



AS101

(0.5 µg/ml)

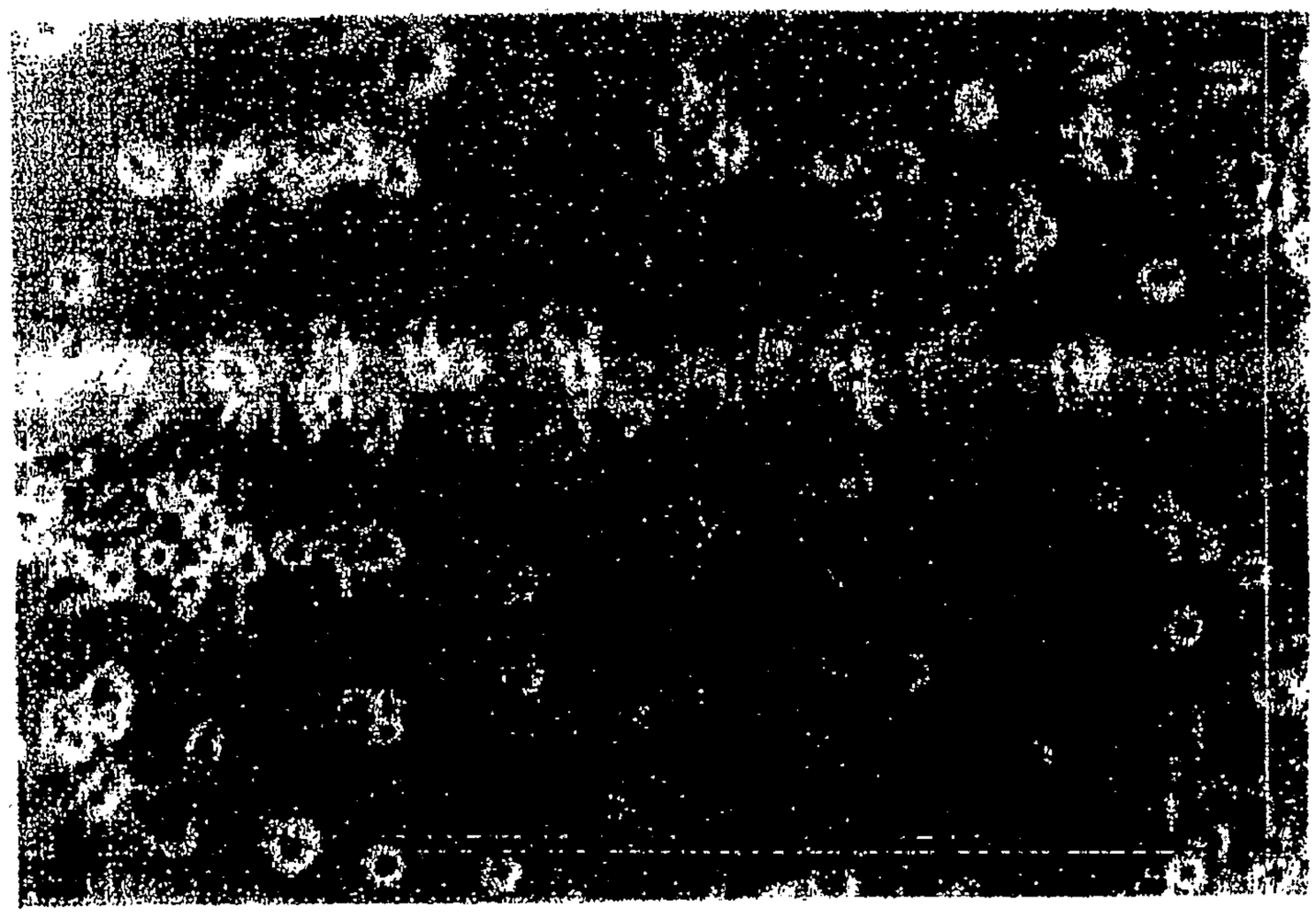


NGF

(100ng/ml)

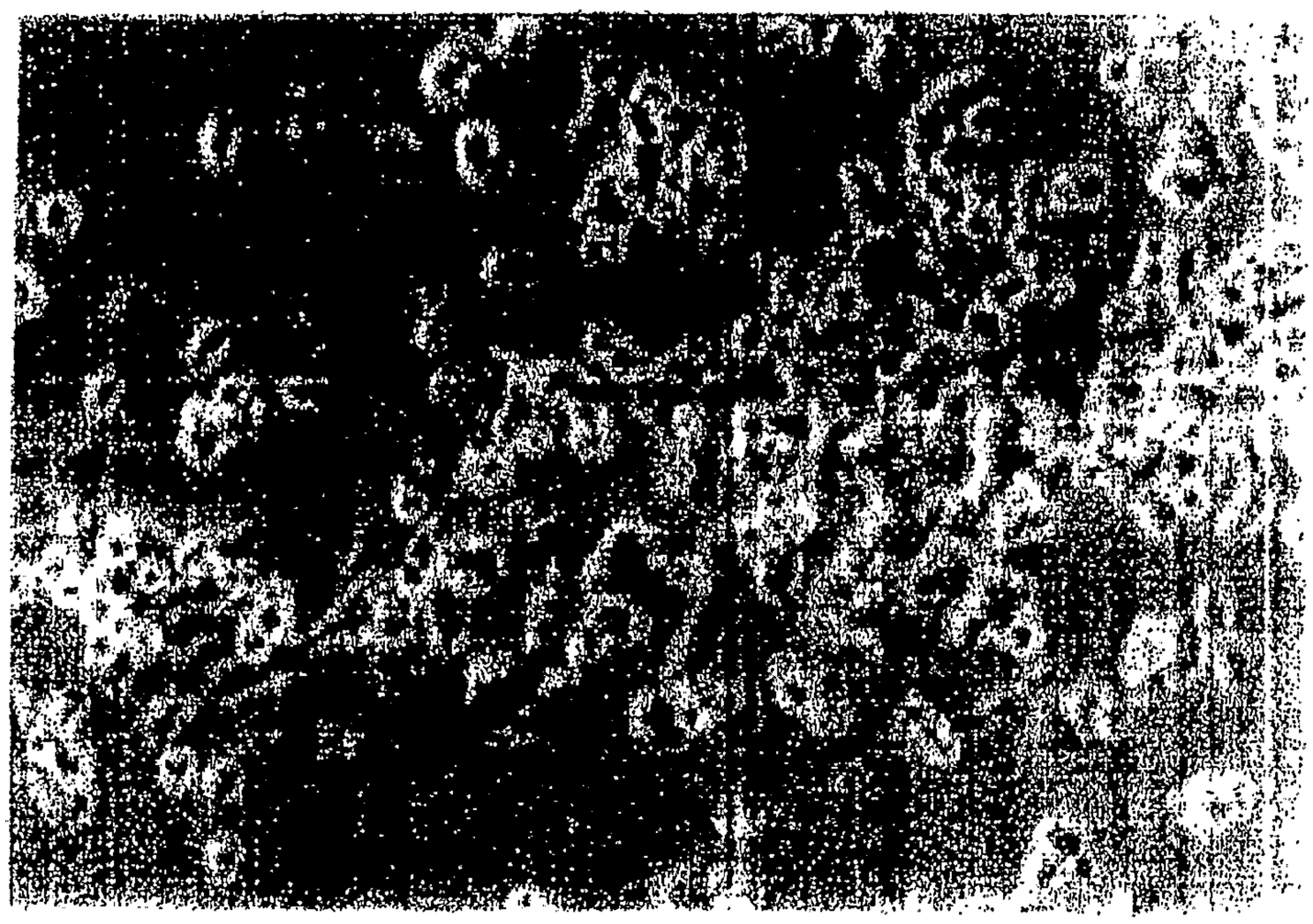
Fig. 3c CYS118

CONTROL



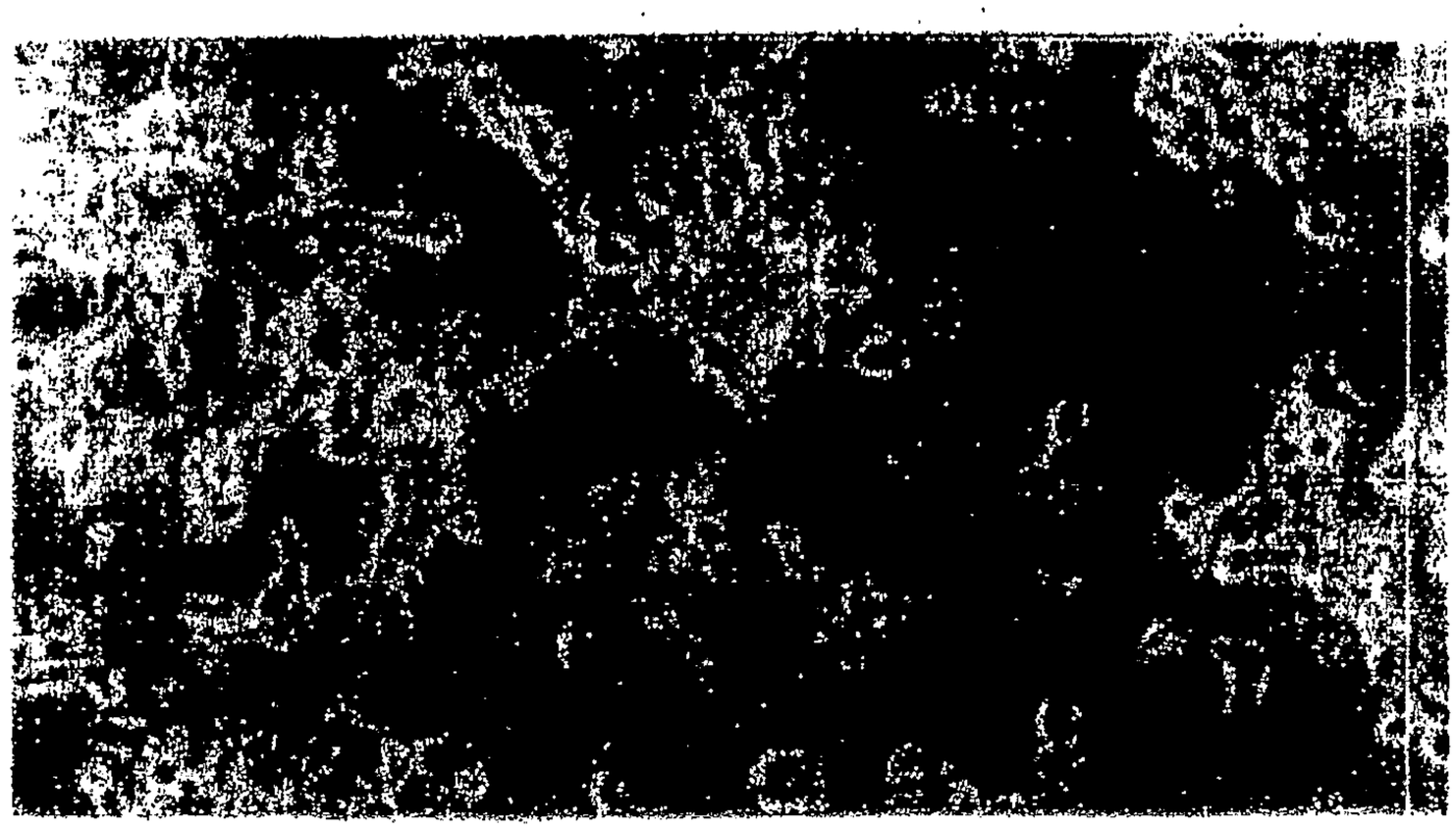
AS101

(0.5 µg/ml)



NGF

(100ng/ml)



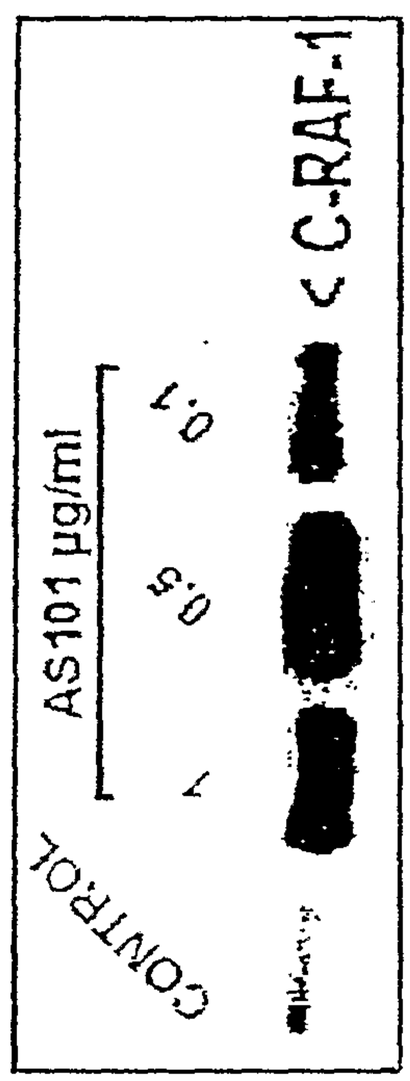


Fig. 4 Cells Incubated With AS101 for 15 minutes

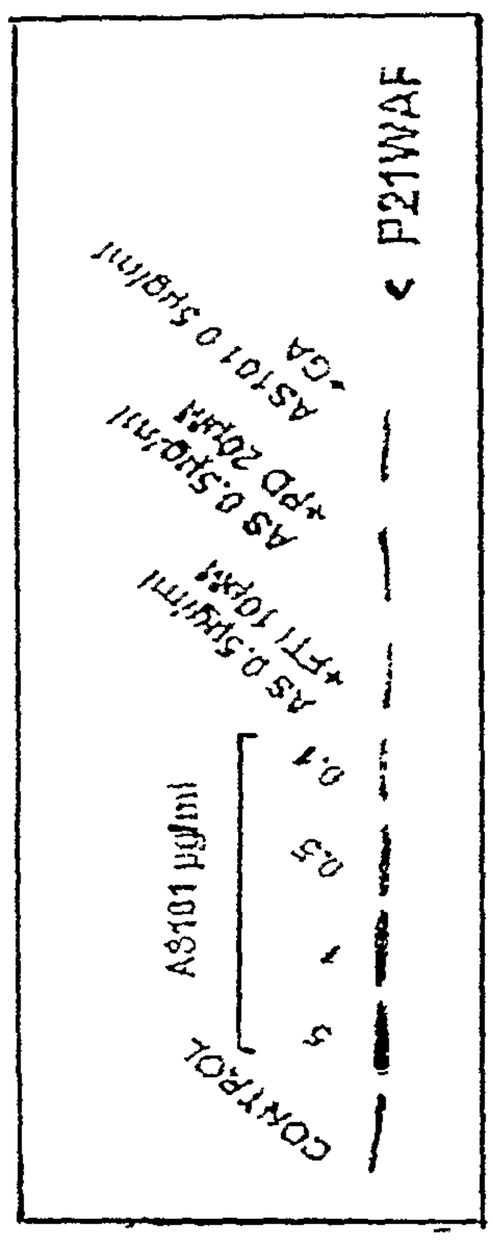


Fig. 5 Cells Incubated With AS101 for 24 hours