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Our proposed research plan involves laboratory studies using an in vivo severe combined immunodeficiency (SCID) mouse model of human metastatic breast cancer as well as cynomologous monkeys to examine the anti-breast cancer activity, toxicity, and pharmacodynamic features of EGF-Gen. Our primary goal in the proposed animal studies is to determine whether systemic exposure levels of EGF-Gen found to be therapeutically effective in SCD mouse models of human breast cancer can be achieved in cynomologous monkeys without excessive toxicity. The knowledge gained from these studies is expected to facilitate the design of effective biotherapy and combined biochemotherapy regimes for breast cancer patients. The proposed research is directly relevant to a critical issue in the treatment of breast cancer.

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Principal Investigator's Signature

10-13-98
Date

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INTRODUCTION

We have continued our efforts to optimize the design of the EGF-Genistein and related tyrosine kinase inhibitor conjugates. The goal of these efforts is to prepare a new generation of EGF conjugates with unprecedented activity as well as stability. The design optimization represents work done at the Hughes Institute whereas the mouse and monkey studies are being conducted at the University of Minnesota. The work as well as analyses are ongoing and no conclusions are yet possible as to whether or not the novel EGF conjugates will be superior to the first generation EGF conjugates. Depending on these results, we will pick the most promising conjugate and start its use as part of combined biochemotherapy regimens, as originally proposed in our application.

BODY

SECTION I: DESIGN OPTIMIZATION

MATERIALS AND METHODS

Preparation of EGF-Genistein and Related Conjugates . rhEGF was produced in *E. coli* harboring a genetically engineered plasmid that contains a synthetic gene for human EGF fused at the N-terminus to a hexapeptide leader sequence for optimal protein expression and folding. rhEGF fusion protein precipitated in the form of inclusion bodies and the mature protein was recovered by trypsin-cleavage followed by purification using ion exchange chromatography and HPLC. rhEGF was 99% pure by reverse-phase HPLC and SDS-PAGE with an isoelectric point of 4.6 ± 0.2. The endotoxin level was 0.17 EU/mg.

The recently published photochemical conjugation method using the hetero-bifunctional photoreactive crosslinking agent, Sulfosuccinimidyl 6-[4'azido-2'-nitrophenylamino]hexanoate (Sulfo-SANPAH) (Pierce Chemical Co., Rockford, IL) was initially employed in the synthesis of the EGF-Genistein(Gen) conjugates. Sulfo-SANPAH was dissolved in DMSO and used to modify EGF at molar ratios of 1:1 - 1:10, EGF to crosslinker. Following size-exclusion chromatography to remove unreacted crosslinker and small molecular weight reaction products, the modified rhEGF was mixed with a 10:1 or 20:1 molar ratio of Gen (LC Laboratories, Woburn, MA) [50 mM solution in dimethyl sulfoxide (DMSO)] and then irradiated for 10 - 15 min with long-wave UV light (366 nm Model UVGL-58 Mineralight; UVP, Upland, CA). Photolytic generation of a reactive singlet nitrene on the other terminus of EGF-SANPAH in the presence of a molar excess of Genistein resulted in the attachment of Gen to lysine 28, lysine 48, or the N-terminal residue of EGF. Excess Gen in the reaction mixture was removed by passage through a G25-Sephadex pre-packed column, and 12 kDa EGF-EGF homoconjugates with or without conjugated Gen, as well as higher molecular weight reaction products, were removed by size-exclusion high-performance liquid chromatography (HPLC, Beckman System Gold).

In addition to Sulfo-SANPAH, we also used the following crosslinking agents obtained from Pierce Chemical Company: N-5-azido-2-nitrobenzoyloxysuccinimide(ANB-NOS), Sulfosuccinimidyl 2-[m-azido-o-nitrobenzamido]ethyl-1,3'-dithiopropionate(SAND), and Sulfosuccinimidyl(perfluoroazidobenzamido)ethyl-1,3-dithiopropionate(SFAD). These crosslinkers are of different chain lengths, ANB-NOS being the shortest at 7.7 A, and all have a phenyl azide at one end to react with Genistein following photolysis. The other end of the crosslinker contains an N-hyrodroxysuccinimide ester to react with protein amino groups. SAND and SFAD are cleavable by thiols. We have also used p-

azidophenylglyoxal monohydrate(APG)(9.3 A) as an arginine and photoreactive crosslinking agent.

To avoid exposing EGF to the possible harmful effects of UV light, we have also photolyzed the crosslinker-Genistein mixture prior to the addition of EGF. We dissolved both the crosslinker and Genistein in DMSO and mixed them together using a 20:1, 10:1, or 2.5:1 molar ratio of Genistein to crosslinker. Photolysis was performed at room temperature for periods of time from 15 - 60 minutes using either a Model UVM-57(302 nm mid-range wavelength) or Model UVGL-58(366 nm longwave) UV lamp from UVP(Upland, CA). Following photolysis, the mixture was added to a solution of EGF(in PBS) at a molar ratio of 10:1, crosslinker:EGF in a maximum final DMSO concentration of 10%.

In an effort to generate more potent EGF conjugates, we have also used two Genistein analogues, DDE24 and DDE41, which have themselves been shown to possess cytotoxic activity in in vitro systems. These compounds have been modified to contain an N-hydroxysuccinimide ester for direct conjugation to EGF in the absence of photolysis.

HPLC Analysis. Reverse phase HPLC using a Hewlett-Packard (HP) 1100 series HPLC instrument was used to monitor and characterize the EGF-Gen conjugation. Analytical HPLC was performed using a Spherisorb ODS-2 reverse phase column (250x4 mm, Hewlett-Packard). Prior to the HPLC runs, a Beckman DU 640B spectrophotometer was used to generate a UV spectrum for each of the samples to ascertain the λmax for EGF-Gen, modified and unmodified EGF. Each HPLC chromatogram was subsequently run at wavelengths of 220, 280, and 480 or 308 nm using the multiple wavelength detector option supplied with the instrument to ensure optimal detection of the individual peaks in the chromatogram. Five - 100

uL samples were applied to the above column and analysis was achieved using a gradient flow consisting of 20% to 100% eluent D in a time interval of 0 to 50 min. Eluent A consisted of a mixture of 0.1% trifluoroacetic acid(TFA) in water and eluent D contained 80% acetonitrile (CH3CN), 20% H_2O, and 0.1% TFA.

Size-exclusion chromatography was carried out using a Beckman System Gold Instrument equipped with a TSKG3000SW column. The column was equilibrated in 100 mM sodium phosphate buffer, pH 6.8 at a flow rate of 3 mL/minute.

Mass Spectrometry. Mass spectrometric analysis was routinely performed to determine the relative molecular weights of the modified EGF and EGF-Genistein conjugates. A Hewlett-Packard Model G2025A matrix-assisted laser desorption/ionization mass spectrometer with linear time-of-flight mode (MALDI-TOF). In conjunction with the Hewlett-Packard instrument were a sample preparation assembly model G2024A including a high vacuum pump and a Dos-Chem station controller model G1030A. Before starting the experiment, the instrument was calibrated with protein standards G2025A supplied by Hewlett-Packard; mass calibration was used by peak centroiding at the 80% level. Sinnapinic acid(Hewlett-Packard) was used as a matrix source. Samples were prepared by spotting 1 uL of a mixture of protein, in phosphate buffer, with the matrix solution(1:1, v/v) on the gold surface of the probe with subsequent evaporation under vacuum. Ionization was accomplished with a laser radiating at a 337-nm wavelength(5 ns pulses, laser energy 1.97 uJ) in both single shot and multiple shot modes. The analyzer was used in the linear mode at an accelerating voltage of 28 kV. The obtained spectra represent the sum of consecutive laser shots and have not been smoothed.

SDS-PAGE Analysis. SDS-PAGE was used to monitor the preparation and purification of the EGF-Genistein conjugates. 15% tricine running gels were stained with Coomassie Blue to visualize the protein bands.

Breast Cancer Cells. MDA-MB-231 (ATCC HTB-26) is an EGF-R positive breast cancer cell line initiated from anaplastic carcinoma cells of a 51 year old patient. BT-20 (ATCC HTB-19) is another EGF-R positive breast cancer cell line isolated from the primary breast tumor of a 74 year old patient with grade II mammary adenocarcinoma. MDA-MB-231 cells are cultured in Leibovitz's L-15 medium plus glutamine; BT-20 breast cancer cells are maintained in MEM medium containing 0.1 mM NEAA and Earle's BSS. Both media are further supplemented with 10 % fetal bovine serum. For subculturing, medium is removed from the flasks containing a confluent layer of cells and fresh 0.25% trypsin added for 1-2 min. Trypsin is removed and cultures incubated for 5-10 min at 37^{o}C until the cells detached. Fresh medium is then added and the cells aspirated and dispensed into new flasks.

Cytotoxic Activity of EGF-Genistein and Related EGFConjugates. The specific cytotoxic activity of the EGF-Genistein conjugates is determined initially using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay (Boehringer Mannheim Corp., Indianapolis, IN). Briefly, exponentially growing breast cancer cells are seeded into a 96-well plate at a density of 2.0x 10^4 cells/well and incubated for 24 hr at 37°C prior to drug exposure. On the day of treatment, culture medium is carefully aspirated from the wells and replaced with fresh medium containing the EGF-Genistein conjugates or unconjugated EGF. Triplicate wells were used for each treatment. The cells were incubated with the various compounds for 48 - 72 hours at 37°C in a humidified 5% CO_2 atmosphere(BT-20 cells; MDA-MB-231 cells are incubated in the absence of CO_2). To each well, 10 µl of MTT (0.5 mg/ml final concentration) was

added and the plates incubated at 37°C for 4 hours to allow MTT to form formazan crystals by reacting with metabolically active cells . The formazan crystals were solubilized for a minimum of 4 hr at 37°C in a solution containing 10% SDS in 0.01 M HCl. The absorbance of each well is measured in a microplate reader (Labsystems) at 540 nm. The absorbance is a measure of cell viability; the greater the absorbance the greater the cell viability.

Colony Assays. After overnight treatment with EGF-Gen or PBS, cells were resuspended in clonogenic medium consisting of alpha-MEM supplemented with 0.9% methylcellulose, 30% fetal bovine serum, and 50 μM 2-mercaptoethanol. Cells were plated in duplicate Petri dishes at 100,000 cells/mL/dish and cultured in a humidified 5% CO2 incubator for 7 days. Cancer cell colonies were enumerated on a grid using an inverted phase microscope of high optical resolution. Results were expressed as % inhibition of clonogenic cells at a particular concentration of the test agent using the formula: % Inhibition = (1 - Mean # of colonies [Test] / Mean # of colonies [Control]) x 100.

RESULTS AND DISCUSSION

Our initial EGF-Genistein conjugates were formed using Sulfo-SANPAH as the photolabile crosslinker. We used MALDI-TOF mass spectrometry as a means of monitoring the modification of EGF using different molar ratios of crosslinker to EGF. **Figure 1** shows an example of these results which indicate that very little unmodified EGF(mass of 6200 daltons) remained when 7.5:1 or 10:1 molar ratios were used. In subsequent experiments EGF was modified using a 10:1 molar ratio of Sulfo-SANPAH followed by photolysis in the presence of longwave UV and a 10 - 20-fold molar excess of Genistein. Size-exclusion HPLC revealed the presence of high-molecular weight material and SDS-

PAGE showed the presence of EGF multimers (**Figure 2**).
We also noted that this EGF conjugate precipitated out of solution during short-term storage at 4° C or when frozen for longer periods of time further reducing the yield of the active EGF - Gen conjugate.

Photolyzing the SANPAH-modified EGF at high protein concentrations appeared to be causing the formation of EGF-EGF multimers and denaturing the EGF so we carried out photolysis on the Sulfo-SANPAH-Genistein mixture(in DMSO) prior to the addition of the EGF. This "pre-photolysis" mixture contained a 20:1 molar excess of Genistein to increase the opportunity for the active nitrene to link to Genistein rather than to another SANPAH or EGF molecule. EGF was added to this mixture following photolysis and unreacted SANPAH and Genistein were removed using G-25 Sephadex column chromatography. Size-exclusion HPLC analysis revealed the presence of high molecular weight aggregates. eluting from 35 - 45 minutes post-injection (**Figure 3 B**). Unmodified EGF typically elutes in this system at 58 - 62 minutes (**Figure 3A**). We observed less aggregation if a 2:1 instead of a 4:1 molar ratio of pre-photolyzed SANPAH - Genistein is used to modify EGF(**Figure 3C**),

We then substituted shorter chain-length and less hydrophobic crosslinkers for SANPAH in order to reduce aggregation due to protein-protein hydrophobic interactions. The short-chain crosslinker, ANB-NOS, results in less precipitation/aggregation than was seen using Sulfo-SANPAH. Since Genistein is relatively insoluble in aqueous solutions, we carried out the pre-photolysis using a 2.5:1 or 10:1 molar ratio of Genistein to crosslinker and a 10:1 ratio of crosslinker-Genistein to EGF. The final DMSO concentration was maintained at 10%.
Figure 4A,B shows an initial size-exclusion HPLC purification of EGF-ANB-NOS-Gen conjugates prepared using the above ratios and 15

minutes of longwave UV photolysis. Less aggregation has occurred when the 10:1 ratio is compared to the 2.5:1 ratio and both are significantly less when the ANB-NOS-Gen mixture is pre-photolyzed than when ANB-NOS-modified EGF is mixed with Genistein and then exposed to UV(**Figure 4C**). The SDS-PAGE gel shown in **Figure 5** also indicates that only small amounts of EGF multimers are formed under these conjugation conditions and that size-exclusion HPLC can be used to remove the aggregates.

All of the EGF-ANB-NOS-Gen conjugates possessed some activity in the MTT assay when tested against the BT-20 and/or MDA-MB231 breast cancer cell lines(**Figure 4**). The HPLC-purified 10:1, 10:1 pre-photolyzed conjugate was the most active exhibiting maximum inhibition at a concentration of less than 1 ug/mL.

Figure 6 shows size-exclusion HPLC profiles that were obtained for EGF conjugates prepared using 10:1 ratios of the Genistein analogs, DDE24 and DDE41. These compounds contain an NHS ester and were directly linked to EGF in PBS buffer without photolysis. The EGF-DDE41 conjugate(**A**) appeared to contain more aggregated protein than the EGF-DDE24 conjugate(**B**). The HPLC-semipurified EGF-DDE41 conjugate did appear to have some inhibitory activity in the MTT assay (**Figure 6**).

SECTION II. ANIMAL STUDIES

MATERIALS AND METHODS

The detailed procedures for murine and primate toxicity studies were detailed in the original grant application and also reported in the previously submitted manuscripts regarding the animal toxicity of the first generation EGF conjugates.

RESULTS AND DISCUSSION

We have examined the toxicity of EGF SANPAH conjugates of DDE-24 and DDE-41 as well as EGF ANB-NOS conjugates of Genistein, DDE-24, and DDE-41. As shown in **Figure 7**, no toxicity and no fatalities were observed with any of these treatments. A detailed report of the histopathological study is enclosed as **Appendix 1**. No test article related histologic lesions were found in any of the mice treated with our new generation EGF conjugates.

We have next examined the toxicity of EGF-ANB-NOS-Genistein and EGF-ANB-NOS-DDE41 (EGF-41) in cynomolgus monkeys. Both agents were well tolerated by monkeys. A detailed report of the clinical findings and raw data is enclosed as **Appendix 2**. The monkeys treated with EGF-41 have been sacrificed and the monkeys treated with EGF-ANB-NOS-Genistein will be sacrificed on October 13, 1998 and a detailed histopathology report will be submitted after the analysis of the tissues. The blood samples collected for pharmacokinetic studies have been frozen for future analysis.

Figure Legends

Figure 1 - Figure 1 shows the results of MALDI-TOF mass spectrometry of EGF and modified EGF preparations. The relative abundance of various molecular species are indicated for unmodified EGF, EGF modified with 1:1 - 1:10 molar ratios of Sulfo-SANPAH, and EGF modified with a 1:10 ratio of ANB-NOS.

Figure 2 - Figure 2 is a 15% tricine SDS-PAGE running gel stained with Coomassie Blue to show unmodified EGF and a partially purified EGF-Genistein conjugate prepared by photolyzing SANPAH-modified EGF in the presence of Genistein. Multimers of EGF can be seen in the lanes containing higher amounts of EGF-Genistein .

Figure 3 - Figure 3 shows examples of size-exclusion HPLC profiles of unmodified EGF(**A**), an EGF-Genistein conjugate(prepared using a 1:4 ratio(**B**) and a 1:2 ratio(**C**) of EGF to SANPAH and a pre-photolysis mixture with a 20-fold excess of Genistein to SANPAH). The Beckman System Gold HPLC was equipped with a TSKG3000SW column equilibrated in 100 mM sodium phosphate buffer, pH 6.8, at a flow rate of 3 mL/minute.

Figure 4 - Figure 4 shows HPLC patterns of EGF-Genistein conjugates prepared using the ANB-NOS crosslinker at a 1:10 ratio of EGF to crosslinker and a 2.5:1 ratio(**A**) or a 10:1 ratio(**B**) of Genistein to ANB-NOS in the pre-photolysis mixture. Figure 4**C** shows the HPLC pattern for an EGF-ANB-NOS-Gen conjugate prepared by photolyzing the modified EGF in the presence of a 20-fold excess of Genistein. Results of MTT assays are also presented for the various EGF-Genistein conjugates.

Figure 5 - Figure 5 shows a 15% tricine SDS-PAGE running gel stained with Coomassie Blue to show the initial size-exclusion HPLC purification of an EGF-ANB-NOS-Gen conjugate prepared using 10:1 ratios of Genistein to

ANB-NOS(the pre-photolysis mixture was then irradiated with longwave UV for 60 minutes at room temperature)and ANB-NOS to EGF.

Figure 6 - Figure 6 shows size-exclusion HPLC profiles of the EGF-DDE41(**A**) and EGF-DDE24(**B**) conjugates. High molecular weight aggregates are seen eluting from 34 - 44 minutes in the EGF-DDE41 pattern while the EGF-DDE24 preparation has very little of this material. MTT assay results are included for the HPLC-semi-purified EGF-DDE41 conjugate.

Figure 7 - These figures show the proportion of mice alive after treatment with the various EGF conjugates. The 100% survival observed for each treatment protocol demonstrates that none of these new generation EGF conjugates are toxic to mice.

Appendix I

Histopathologic Evaluation of Tissues from BALB/C Mice on an Intraperitoneal Toxicity Study of EGF-Conjugates:

EGF/24
EGF/41
EGF/ANB-NOS-24
EGF/ANB-NOS-41
EGF/ANB-NOS-GEN

Experiment Dates:
8/4/98

Date: 10/9/98

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Histopathologic Evaluation of Tissues from BALB/C Mice on an Intraperitoneal Toxicity Study of EGF-Conjugates (EGF/24, EGF/41, EGF/ANB-NOS-24, EGF/ANB-NOS-41, and EGF/ANB-NOS-GEN); Experiment Dates 8/4/98.

A. MATERIAL AND METHODS:

1. The study was performed as follows:

a. **EGF-Conjugates:**

1. **EGF/24**:

a. **GROUP 1:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/24, 100 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

b. **GROUP 2:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/24, 200 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

c. **GROUP 3:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/24, 400 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

d. **GROUP 4:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/24, 800 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

2. **EGF/41**:

a. **GROUP 5:** On 8/4/98, 2 female BALB/C mice received a single IP (intravenous) injection of EGF/41, 100 µg, in 200 µl PBS (phosphate buffered saline). Both mice were euthanized clinically healthy on day 30 (9/3/98).

b. **GROUP 6:** On 8/4/98, 2 female BALB/C mice received a single IP (intravenous) injection of EGF/41, 200 µg, in 200 µl PBS (phosphate buffered saline). Both mice were euthanized clinically healthy on day 30 (9/3/98).

3. **EGF/ANB-NOS-24**:

   a. **GROUP 7:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/ANB-NOS-24, 100 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

   b. **GROUP 8:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/ANB-NOS-24, 200 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

4. **EGF/ANB-NOS-41**:

   a. **GROUP 9:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/ANB-NOS-41, 100 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

   b. **GROUP 10:** On 8/4/98, 2 female BALB/C mice received a single IP (intravenous) injection of EGF/ANB-NOS-41, 200 µg, in 200 µl PBS (phosphate buffered saline). Both mice were euthanized clinically healthy on day 30 (9/3/98).

5. **EGF/ANB-NOS-GEN**:

   a. **GROUP 11:** On 8/4/98, 2 female BALB/C mice received a single IP (intravenous) injection of EGF/ANB-NOS-GEN, 100 µg, in 200 µl PBS (phosphate buffered saline). Both mice were euthanized clinically healthy on day 30 (9/3/98).

   b. **GROUP 12:** On 8/4/98, 2 female BALB/C mice received a single IP (intravenous) injection of EGF/ANB-NOS-GEN, 200 µg, in 200 µl PBS (phosphate buffered saline). Both mice were euthanized clinically healthy on day 30 (9/3/98).

   c. **GROUP 13:** On 8/4/98, 3 female BALB/C mice received a single IP (intravenous) injection of EGF/ANB-NOS-GEN, 800 µg, in 200 µl PBS (phosphate buffered saline). All 3 mice were euthanized clinically healthy on day 30 (9/3/98).

b. **PBS Treatment (Control Group):**

1. **GROUP 14:** On 8/4/98, 6 female BALB/C mice received a single IP (intraperitoneal) injection of 200µl PBS (phosphate buffered saline). All 6 mice were euthanized clinically healthy on day 30 (9/3/98).

c. At necropsy, no gross lesions were observed in any group.

2. Clinical Phase, Necropsy and harvesting of tissues:

a. The clinical phase, necropsy and harvesting of tissues was performed at the Wayne Hughes Institute Pre-Clinical Laboratory, 2680 Patton Road, Roseville, MN 55113.

b. At death, all mice had routine postmortem examinations. All tissues were collected, fixed in 10% formalin, and processed for histologic sectioning in a routine manner.

c. The histology slides were stained with Hematoxylin and Eosin.

d. The histologic evaluation of the tissues and report compilation was done by B.Waurzyniak, DVM., MS., (veterinary pathologist).

Histopathologic Evaluation of Tissues from BALB/C Mice on an Intraperitoneal Toxicity Study of EGF-Conjugates (EGF/24, EGF/41, EGF/ANB-NOS-24, EGF/ANB-NOS-41, and EGF/ANB-NOS-GEN); Experiment Dates 8/4/98.

B. RESULTS:

1. **TABLE 1: Treatment Table and Mouse Identification**..... **8/4/98**

TREATMENT:	EGF/24				EGF/41		EGF/ANB-NOS-24		EGF/ANB-NOS-41		EGF/ANB-NOS-GEN			PBS
GROUP:	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DOSAGE (µg):	100	200	400	800	100	200	100	200	100	200	100	200	800	0
TX ROUTE:	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP	IP
Outcome: 1. SM = euthanized moribund 2. SH = euthanized healthy 3. D = died.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.	All SH.
Experiment duration (days):	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mouse ID Numbers:	19095 19096 19097	19102 19103 19104	19064 19100 19101	19061 19062 19063	19070 10979	19077 19078	19091 19092 19093	19090 19098 19099	19060 19073 19074	19071 19072	19075 19076	19143 19144	19140 19141 19142	19094 19085 19086 19087 19088 19089
# Mice / Group	3	3	3	3	2	2	3	3	3	2	2	2	3	6
# Mice Examined	0	0	0	3	0	2	0	3	0	2	0	0	3	6

4. No test agent related lesions were found in any mice in this study.

5. Incidental findings: (see Table 2).

   a. Epicarditis, mild, nonsuppurative, focal, chronic was present in:

      1. 1/3 (33%) of mice from Group 4 (EGF/24 800 µg);

      2. 1/3 (33%) of mice from Group 8 (EGF/ANB-NOS-24 200 µg);

      3. 1/6 (17%) of mice from Group 14 (PBS - Control).

   b. Epicardial dystrophic mineralization, mild, chronic, was present in:

      1. 1/3 (33%) of mice from Group 4 (EGF/24 800 µg);

      2. 1/3 (33%) of mice from Group 8 (EGF/ANB-NOS-24 200 µg);

      3. 1/3 (33%) of mice from Group 13 (EGF/ANB-NOS-GEN 800 µg);

      4. 1/6 (17%) of mice from Group 14 (PBS - Control).

c. Hepatitis, multifocal, mild, subacute, was present in:

1. 1/3 (33%) of mice from Group 4 (EGF/24 800 µg);

2. 1/2 (50%) of mice from Group 6 (EGF/41 200 µg);

3. 1/6 (17%) of mice from Group 14 (PBS - Control).

d. Gastritis, mild, focal, non-ulcerative, subacute, was present in:

1. 1/3 (33%) of mice from Group 4 (EGF/24 800 µg);

2. 1/6 (17%) of mice from Group 14 (PBS - Control).

e. Dystrophic mineralization, of the gastric tunica muscularis, focal, mild, chronic, was present in:

1. 1/3 (33%) of mice from Group 8 (EGF/ANB-NOS-24 200 µg);

2. 1/2 (50%) of mice from Group 10 (EGF/ANB-NOS-41 200 µg);

3. 2/3 (67%) of mice from Group 13 (EGF/ANB-NOS-GEN 800 µg);

4. 1/6 (17%) of mice from Group 14 (PBS - Control).

C. COMMENTS:

1. The EGF-Conjugates (EGF/24, EGF/41, EGF/ANB-NOS-24, EGF/ANB-NOS-41, and EGF/ANB-NOS-GEN) were non-toxic under the conditions of this study. All mice were euthanized clinically healthy at the end of the study.

2. Histologic lesions related to (IP) EGF-Conjugates (EGF/24, EGF/41, EGF/ANB-NOS-24, EGF/ANB-NOS-41, and EGF/ANB-NOS-GEN) were not present in any mice in the study.

Histopathologic Evaluation of Tissues from BALB/C Mice on an Intraperitoneal Toxicity Study of EGF-Conjugates (EGF/24, EGF/41, EGF/ANB-NOS-24, EGF/ANB-NOS-41, and EGF/ANB-NOS-GEN); Experiment Dates 8/4/98.

TABLE 2: Histopathologic Evaluation of Tissues from BALB/C Mice on a Toxicity Study of EGF-Conjugates (Experiment Date 8/4/98).

Group Number:	4	6	8	10	13	14
Treatment:	EGF/24	EGF/41	EGF/ANB-NOS-24	EGF/ANB-NOS-41	EGF/ANB-NOS-GEN	PBS
Treatment Dose (μg):	800	200	200	200	800	0
Total Number of Mice / Group:	3	2	3	2	3	6
Total # of Mice with Histology Examination:	3	2	3	2	3	6
Tissue/Diagnosis/ Modifier(S):						
Bone:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Bone Marrow:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Brain:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Heart:						
1. WNL	1	2	1	2	2	4
2. NE	0	0	0	0	0	0
3. Epicarditis, nonsuppurative, mild, focal, chronic	1 (33%)	0	1 (33%)	0	0	1 (17%)
4. Dystrophic mineralization, epicardium, ± fibrosis, mild, focal - multifocal, chronic	1 (33%)	0	1 (33%)	0	1 (33%)	1 (17%)
Kidney:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Large Intestine:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Liver:						
1. WNL	2	1	3	2	3	5
2. NE	0	0	0	0	0	0
3. Hepatitis, multifocal, mixed inflammation, mild, subacute ± focal hepatic necrosis	1 (33%)	1 (50%)	0	0	0	1 (17%)
Lung:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Lymph node:						
1. WNL	3	1	0	1	2	2
2. NE	0	1	3	1	1	4
Ovary:						
1. WNL	1	1	2	1	1	2
2. NE	2	1	1	1	2	4
Pancreas:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0

Histopathologic Evaluation of Tissues from BALB/C Mice on an Intraperitoneal Toxicity Study of EGF-Conjugates (EGF/24, EGF/41, EGF/ANB-NOS-24, EGF/ANB-NOS-41, and EGF/ANB-NOS-GEN); Experiment Dates 8/4/98.

TABLE 4: (Continued).

Group Number:	**4**	**6**	**8**	**10**	**13**	**14**
Treatment:	EGF/24	EGF/41	EGF/ANB-NOS-24	EGF/ANB-NOS-41	EGF/ANB-NOS-GEN	PBS
Treatment Dose (μg):	**800**	**200**	**200**	**200**	**800**	**0**
Skeletal Muscle:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Skin:						
1. WNL	3	2	3	1	2	6
2. NE	0	0	0	1	0	0
Small Intestine:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Spinal cord:						
1. WNL	0	0	0	0	1	3
2. NE	3	2	3	2	2	3
Spleen:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Stomach:						
1. WNL	2	2	2	1	1	4
2. NE	0	0	0	0	0	0
3. Gastritis, mixed inflammation, mild, focal, non-ulcerative, subacute	1 (33%)	0	0	0	0	1 (17%)
4. Dystrophic mineralization, focal, mild, chronic, tunica muscularis.	0	0	1 (33%)	1 (50%)	2 (67%)	1 (17%)
Thymus:						
1. WNL	3	2	3	2	3	6
2. NE	0	0	0	0	0	0
Urinary Bladder:						
1. WNL	3	1	2	1	2	4
2. NE	0	1	1	1	1	2
Uterus:						
1. WNL	3	2	3	2	2	6
2. NE	0	0	0	0	1	0

WNL = within normal limits. NE = not examined.
% = (number of mice with lesion ÷ total number of mice examined) x 100

Appendix II

Monkey 68J EGF/ANB-NOS-Gen Summary

On 9/29/98, day 1 of this study, Monkey 68J, a female adult cynomologus macaque, was given a 25ml bolus of 5mg EGF/ANB-NOS-Gen intravenously over a two minute time period. A pre infusion pharmacology sample was taken as well as blood for chemistry, CBC, and coagulation tests. A non-sterile urinalysis was also done. Time points for blood draws for the pharmacology were: 10min, 20min, 30min, 1hour, 2hour, 4hour, 8hour, and 12hour. Pharmacology sample were taken every day up to the one week sample and a two week sample was also taken.

Vitals, chemistries, and CBCs were taken on days 1-10 and 15. Coagulation tests were taken on days 1, 4, 7, 10, and 15. A second urinalysis was taken on day 15. Clinical observations are detailed in the attached data forms.

The sacrifice date for this monkey is 10/13/98.

Monkey 68N EGF/ANB-NOS-Gen Summary

On 9/29/98, day 1 of the study, Monkey 68N, a female adult cynomologus macaque weighing 3.9 kg, was given a 25ml bolus of 1mg EGF/ANB-NOS-Gen intravenouly over a two minute time period. A pre infusion pharmacology sample was taken as well as blood for chemistry, CBC, and coagulation tests. A non-sterile urinalysis was also done. Time points for blood draws for the pharmacology were: 10min, 20min, 30min, 1hour, 2hour, 4hour, 8hour, and 12hour. Pharmacology samples were taken every day up to the one week sample and a two week sample was also taken.

Vitals, chemistries, and CBCs were taken on days 1-10 and 15. Coagulation tests were taken on days 1, 4, 7, 10, and 15. A second urinalysis was taken on day 15. Clinical observations are detailed in the attached data forms.

Sacrifice date for this monkey is 10/13/98.

Toxicity of EGF/ANB-NOS-Gen in Cynomolgus Monkeys

System	Grade of Maximum Toxicity (Time)	
	68N (1 mg, Bolus, IV)	68J (5 mg, Bolus, IV)
Activity/Feeding	0	0
Fever	0	0
Weight Loss	0	0
Skin (Alopecia)	0	0
Cardiac		
Tachycardia	1	0
Hypertension	NA	NA
Hypotension	NA	NA
Pulmonary		
Clinical	0	0
Respiratory rate	1	0
Renal		
Creatinine	0	0
Electrolytes	0	0
Proteinuria	0	0
Hematuria	0	0

Toxicity of EGF/ANB-NOS-Gen in Cynomolgus Monkeys

System	Grade of Maximum Toxicity (Time)	
	68N (1 mg, Bolus, IV)	68J (5 mg, Bolus, IV)
Liver		
ALT	1	1
Bili	0†	0†
Gastrointestinal		
Nausea/Vomiting	0	0
Diarrhea	0	0
Constipation	0	0
Nervous System		
Central	0	0
Peripheral	0	0
Infection	0	0
Blood		
Leukopenia	0	0
Anemia	2 (d10, 9.5 g/dL*)	2 (d10, 8.6 g/dL*)
Thrombocytopenia	0	0
Metabolic	3 (d1)	2 (d1, 3, 7 - 10)

†Bilirubin elevated due to hemolytic sample.
*Hemoglobin level decreased due to blood draw.

Monkey 68I EGF/41 Summary

On 9/16/98 on day 1 of this study, Monkey 68I, a female adult cynomologus macaque weighing 4.25kg, was given a 25ml bolus of 5mg EGF/41 intravenously during a two minute time period. A pre infusion pharmacology sample was taken as well as blood for chemistry, CBC, and coagulation tests. A non-sterile urinalysis was also done. Time points for blood draws for the pharmacology were: 10min, 20min, 30min, 1hour, 2hour, 4hour, 8hour, and 12hour. Pharmacology samples were also taken every day up to the one week sample and a two week sample was also taken

Vitals, CBCs and chemistries have been taken for days 1-10 and day 15. Coagulation tests were taken on days 1, 4, 7, 10, and 15. Another urinalysis was done on day 15. Clinical observations are detailed on the attached data forms.

This monkey was sacrificed on 9/30/98.

Monkey 68K EGF/41 Summary

On 9/16/98 day 1 of the study, Monkey 68K, an adult female cynomologus macaque, weighing 4.05kg, was given a 25ml bolus of 1mg EGF/41 intravenously over a two minute time period. A pre infusion pharmacology sample was taken as well as blood for chemistry, CBC, and coagulation tests. A non-sterile urinalysis was also done. Time points for blood draws for the pharmacology were: 10min, 20min, 30min, 1hour, 2hour, 4hour, 8hour, and 12hour. Blood was drawn for pharmacology timepoints every day up to the 1 week sample and also a 2 week sample was drawn.

Vitals, chemistries and CBCs were taken on days 1-10 and day 15. Coagulation tests were also taken on days 1, 4, 7, 10, and 15. Clinical observations are detailed on the attached data forms.

This monkey was sacrificed on 9/30/98.

Toxicity of EGF/41 in Cynomolgus Monkeys

System	Grade of Maximum Toxicity (Time)	
	68K (1 mg, Bolus, IV)	**68I** (5 mg, Bolus, IV)
Activity/Feeding	0	3 (d3)
Fever	0	0
Weight Loss	0	0
Skin (Alopecia)	0	0
Cardiac		
Tachycardia	0	0
Hypertension	NA	NA
Hypotension	NA	NA
Pulmonary		
Clinical	0	0
Respiratory rate	0	0
Renal		
Creatinine	0	0
Electrolytes	0	0
Proteinuria	0	0
Hematuria	0	1

Toxicity of EGF/41 in Cynomolgus Monkeys

System	Grade of Maximum Toxicity (Time)	
	68K (1 mg, Bolus, IV)	**68I** (5 mg, Bolus, IV)
Liver		
ALT	0	3 (d15)
Bili	0	0
Gastrointestinal		
Nausea/Vomiting	0	0
Diarrhea	0	0
Constipation	0	0
Nervous System		
Central	0	0
Peripheral	0	0
Infection	0	0
Blood		
Leukopenia	0	0
Anemia	0	2 (d10, 8.5 g/dL*)
Thrombocytopenia	0	0
Metabolic	2 (d1, 3, 6, 8 - 10)	3 (d1)

*Hemoglobin level decreased due to blood draw.

MONKEY
TOXICITY AND COMPLICATIONS CRITERIA

SITE	MEASURE	GRADE				
		WNL	1 (Mild)	2 (Moderate)	3 (Severe)	4(Unaccept)
HEMATOLOGY	1. WBC/ Leukocytosis	4.0 - 14.0 WNL	3.0 - 3.9 14.1 - 20.0	2.0 - 2.9 20.1 - 30.0	1.0 - 1.9 30.1 - 40.0	< 1.0 > 40.0
	2. HgB	> 11.5	10.0 - 11.5	8.0 - 9.9	6.5 - 7.9	< 6.5
	3. PLT	> 150	75.0 - 150.0	50.0 - 74.9	25.0 - 49.9	< 25.0
FEEDING	Feeding Abnormality	none	-----	decreased intake	not eating	dehydration req. IV
GASTROINTEST	Diarrhea	none	mild amt of soft stool	mod amt of soft stool, diarrhea, minimal bleeding, small amt of mucous in stool	watery diarrhea, excessive amt of soft stool, large amt of mucous in stool	bloody diarrhea or severe dehydration due to diarrhea
LIVER	1. Bili	≤ 1.3	1.4 - 1.5	1.6 - 2.0	2.1 - 4.0	> 4.0
	2. ALT	≤ 60	61 - 150	151 - 300	301 - 1200	> 1200
PANCREAS	Amylase	≤ 363	364 - 545	546 - 726	727 - 1815	> 1815
RENAL	1. Urea N	< 20	20 - 39	40 - 59	60 - 79	≥ 80
	2. Creatinine	≤ 1.1	1.2 - 1.5	1.6 - 3.0	3.1 - 6.0	> 6.0
	3. Urine: protein	negative	(1 or more) + 1	(1 or more) + 2 to + 3	(1 or more) + 4	(1 or more) > + 4, marked protein loss
	blood	negative	> 10	see blood	see blood clots	transfusion req. bec of bloody urine
	infection	negative	+ 5 WBC, < 10,000 colonies, (+)	many WBC (++)	sheets of WBC, > 10,000 colonies, (+++) or (++++)	sepsis due to urine dehydr, weight loss, 1.008 - 1.012
	spec. grav.	1.013-1.035	-----	<1.013,>1.035	1.008 - 1.012	-----
PULMONARY	1. Clinical	clear	wheezing	crackle	severe respir distress	-----
	2. Respir Rate:					
	a) conscious	28 - 32	33 - 50	51 - 70	71 - 80	> 80
	b) anesthetized	20 - 32	33 - 50	51 - 70	71 - 80	> 80

SITE	MEASURE	GRADE				
		WNL	1 (Mild)	2 (Moderate)	3 (Severe)	4(Unaccept)
CARDIAC	1. Murmur	none	slight	significant	very significant	-----
	~~	~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~
	2. Heart Rate:					
	a) conscious	195 - 265	160 - 195 265 - 300	125 - 159 301 - 335	< 125 > 335	-----
	b) anesthetized	145 - 195	120 - 145 195 - 220	95 - 119 221 - 245	< 95 > 245	-----
	~~	~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~
	3. Hypertension		(1-2 readings)	(all 3 readings)		
	a) conscious	90 - 130/ 35 - 65	131 - 150/ 66 - 80		> 150/> 80 (req. saline)	-----
	b) anesthetized	45 - 85/ 25 - 45	86 - 105/ 46 - 55		> 105/> 55 (req. saline)	-----
	~~	~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~~~~	~~~~~~~~~
	4. Hypotension		(1-2 readings)	(all 3 readings)		
	a) conscious	90 - 130/ 35 - 65	70 - 89/ 25 - 34		< 70/< 25 (req. saline)	-----
	b) anesthetized	45 - 85/ 25 - 45	25 - 44/ 15 - 24		< 25/< 15 (req. saline)	-----
NEUROLOGY	1. Motor	no change	mild weakness	mod. weakness	severe weakness	paralysis
	2. Examination of Gait	(5) normal strength/ coordination	(4) supportive standing, min. paraparesis/ ataxia	(3) supportive standing, stumbles freq. and falls, mild paraparesis/ ataxia	(2) can't stand, when assisted - stumbles and falls frequently, mod. paraparesis/ ataxia	(1) can't stand, slight movement when held by tail, severe paraparesis (0) para-plegic
	3. CNS	no change	drowsy	lethargic, very drowsy	seizures	comatose
SKIN	1. Allergic	none	mild rash	swelling, hives, itching	generalized swelling, itching, req. treatment	skin sloughing
	2. Alopecia	none	mild localized loss	complete local loss, mild general loss	severe generalized loss	bald
WEIGHT CHANGE	From 1st day	± 2% - 4.9%	± 5% - 9.9%	± 10 %-19.9%	±≥ 20.0%	-----
COAGULATION	1. INR	< 1.09	1.09 - 1.35	1.36 - 1.59	1.6 - 2.1	≥ 2.2
	2. PTT	< 34.0	34.0 - 54.9	55.0 - 79.5	80.0 - 99.9	≥ 100.0
	3. CFIB (elev = infection)	> 0.15	0.11 - 0.15	0.08 - 0.10	0.05 - 0.07	≤ 0.04

SITE	MEASURE	GRADE				
		WNL	1 (Mild)	2 (Moderate)	3 (Severe)	4(Unaccept)
METABOLIC	1. Anion Gap	≤ 16	17 - 22	23 - 30	31 - 35	≥ 36
	2. Glucose	65 - 115	55 - 64 116 - 160	40 - 54 161 - 250	30 - 39 251 - 500	< 30 > 500
	3. Albumin	≥ 3.5	3.0 - 3.49	2.0 - 2.9	1.5 - 1.9	< 1.5
ACTIVITY	1. Overall Activity Level	no symptoms	symptoms, able to carry out daily activities	minimal prodding required	strong prodding required	can't move even with prodding
	2. Hunched/ Discomfort	none	mild	moderate	mod-severe	severe
TEMPERATURE	Fever/ Hypothermia	97° - 101.5°	101.6° - 103°	103.1° - 104°	> 104°, < 98.5° consc, < 97° anesth (not induced)	consistently > 104°, consistently < 97°
INFECTION		none	runny eyes/nose, cough, mild diarrhea	localized skin infection, severe cold, mod. diarrhea, w/o systemic symptoms	positive culture, w/systemic symptoms	life threatening
OVERALL HEALTH	Not including blood results	-----	mild	moderate	severe	deathly sick

Appendix III

NEW DATA* [A.03.00 , #5081]

Figure 1A

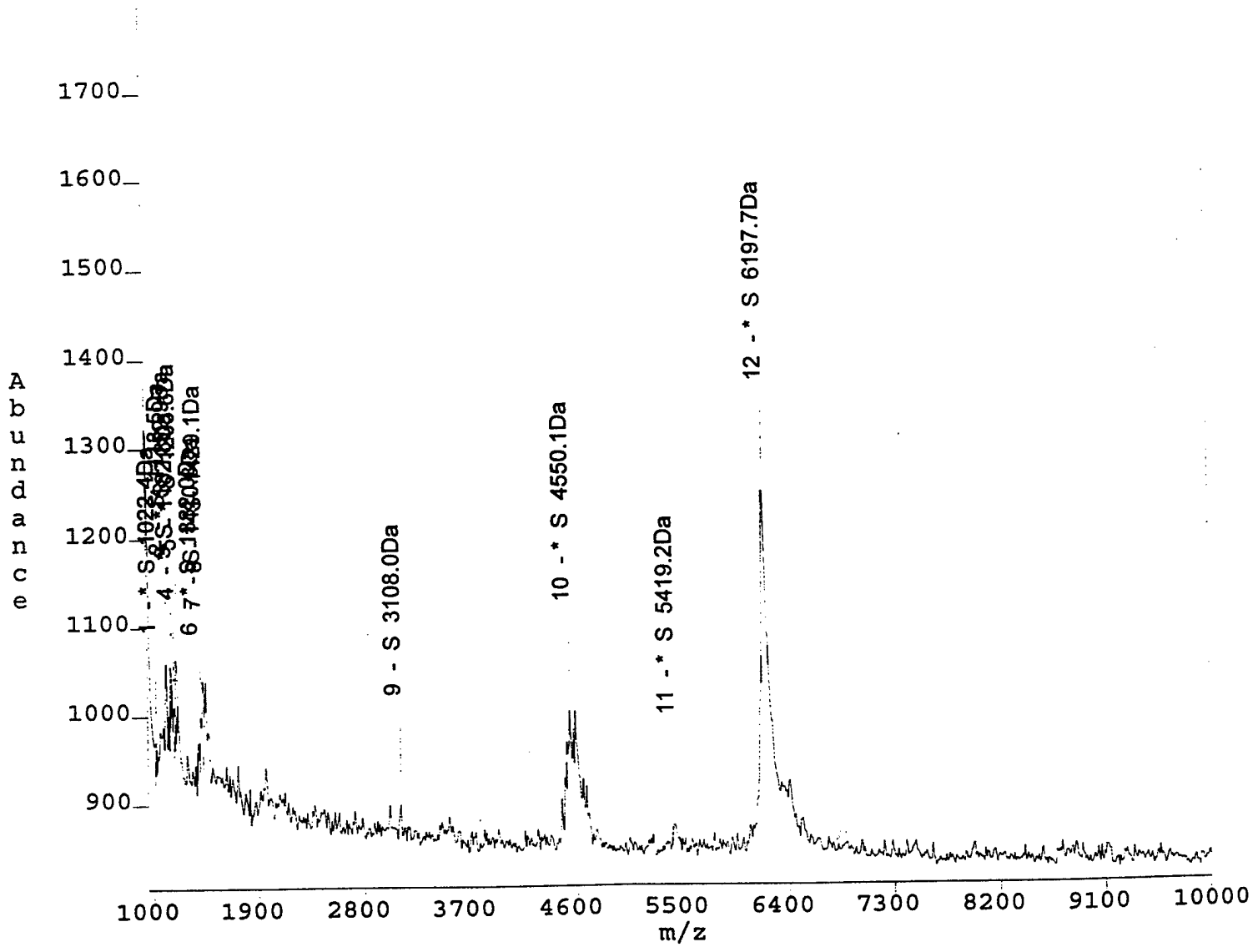
Sample Name	EGF
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

1 mg/10 mL

Collected	Fri Apr 17 10:45:52 1998
Processed	Fri Apr 17 10:48:01 1998
Printed	Fri Apr 17 10:52:41 1998

Sequence			
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET		
Collection Mode	Auto Multi Shots (S/N 28.5) (50 of 136) Mesa 1 [25-82]		
Laser Energy	2.28 (0.55) uJ	Vacuum	1.44e-006 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]



Sign: _____ Date: / /

Figure 1A (cont'd)

NEW DATA* [A.03.00 , #5081]

Sample Name	EGF
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

1 mg/10 mL

Collected	Fri Apr 17 10:45:52 1998
Processed	Fri Apr 17 10:48:01 1998
Printed	Fri Apr 17 10:52:41 1998

Sequence	
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET

Collection Mode	Auto Multi Shots (S/N 28.5) (50 of 136) Mesa 1 [25-82]		
Laser Energy	2.28 (0.55) uJ	Vacuum	1.44e-006 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Peak	Height	Area	MW	delMW	%err	Name	(page 1 of 1)
1 * S	972	903	1022.4				
2 S	1061	1704	1113.5	91.1			
3 * S	1057	1170	1159.9	46.4			
4 * S	1011	892	1182.8	22.9			
5 * S	1065	3920	1205.6	22.7			
6 * S	971	778	1382.0	176.4			
7 S	1000	859	1410.8	28.8			
8 * S	1040	1277	1429.1	18.3			
9 S	899	591	3108.0	1678.9			
10 * S	1003	2400	4550.1	1442.1			
11 * S	873	1005	5419.2	869.0			
12 * S	1246	18962	6197.7	778.5			
13 * S	881	950	12396.7	6199.0			
14 * S	856	604	12546.9	150.2			

*=Gauss, (D)eflected, (C)alibrant/(S)ample, M=Manual, P#=Polymer, ? = changed.

Sign: _____ Date: / /

NEW DATA* [A.03.00 , #5091]

Figure 1B

Sample Name	EGF/SANPAH 1:1
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

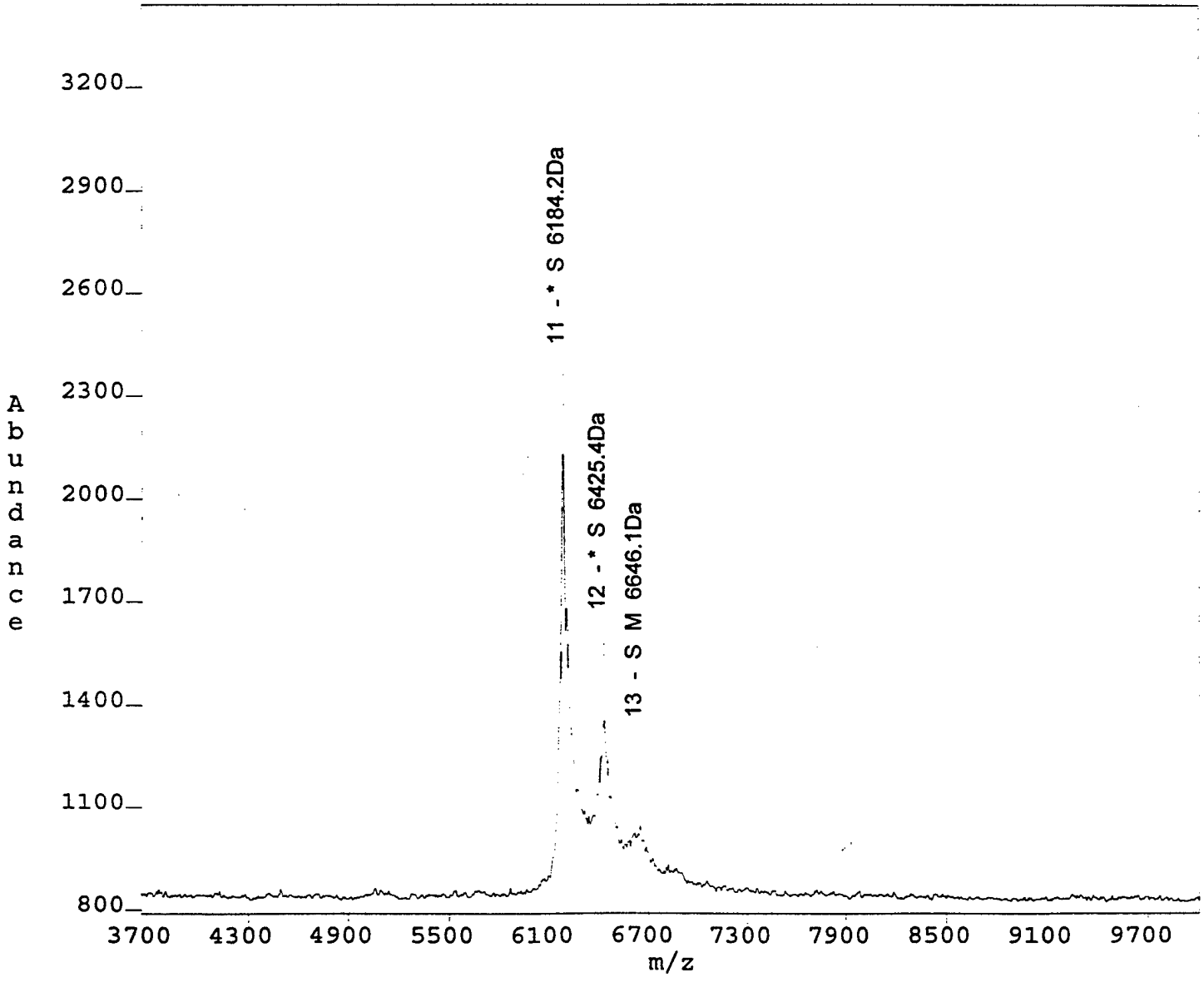
4/14/98

Collected	Fri Apr 17 11:32:24 1998
Processed	Fri Apr 17 11:34:03 1998
Printed	Fri Apr 17 11:34:06 1998

Sequence	
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET

Collection Mode	Auto Multi Shots (S/N 93.0) (50 of 79) Mesa 1 [25-25]		
Laser Energy	1.23 (0.43) uJ	Vacuum	1.80e-006 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]



Sign: _____ Date: / /

Figure 1B (cont'd)

NEW DATA* [A.03.00 , #5091]

Sample Name	EGF/SANPAH 1:1
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

4/14/98

Collected	Fri Apr 17 11:32:24 1998
Processed	Fri Apr 17 11:34:03 1998
Printed	Fri Apr 17 11:34:06 1998

Sequence
Method C:\HPTOFOLD\METHOD\PEP-NEG.MET

Collection Mode Auto Multi Shots (S/N 93.0) (50 of 79) Mesa 1 [25-25]
Laser Energy 1.23 (0.43) uJ Vacuum 1.80e-006 torr

Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Peak			Height	Area	MW	delMW	%err	Name
1	*	S	1024	779	977.3			
2	*	S	951	839	997.2	20.0		
3		S	982	582	1136.5	139.2		
4	*	S	1002	1082	1157.7	21.2		
5	*	S	1038	1370	1178.8	21.2		
6	*	S	1089	1889	1201.3	22.4		
7	*	S	935	628	1382.1	180.8		
8	*	S	991	956	1424.4	42.3		
9	*	S	981	786	1446.2	21.8		
10	*	S	922	651	1695.7	249.5		
11	*	S	2131	46211	6184.2	4488.5		- EGF
12	*	S	1356	5411	6425.4	241.2		- EGF/SAN (1:1)
13		S	M 1047	2764	6646.1	220.7		- EGF (SAN (1:2?)
14	*	S	926	2340	12390.9	5744.8		
15		S	1070	687	18094.9	5704.0		

(page 1 of 1)

*=Gauss, (D)eflected, (C)alibrant/(S)ample, M=Manual, P#=Polymer, ? = changed.

Sign: _____ Date: / /

NEW DATA* [A.03.00 , #5095]

Sample Name	EGF/SANPAH 1:5
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

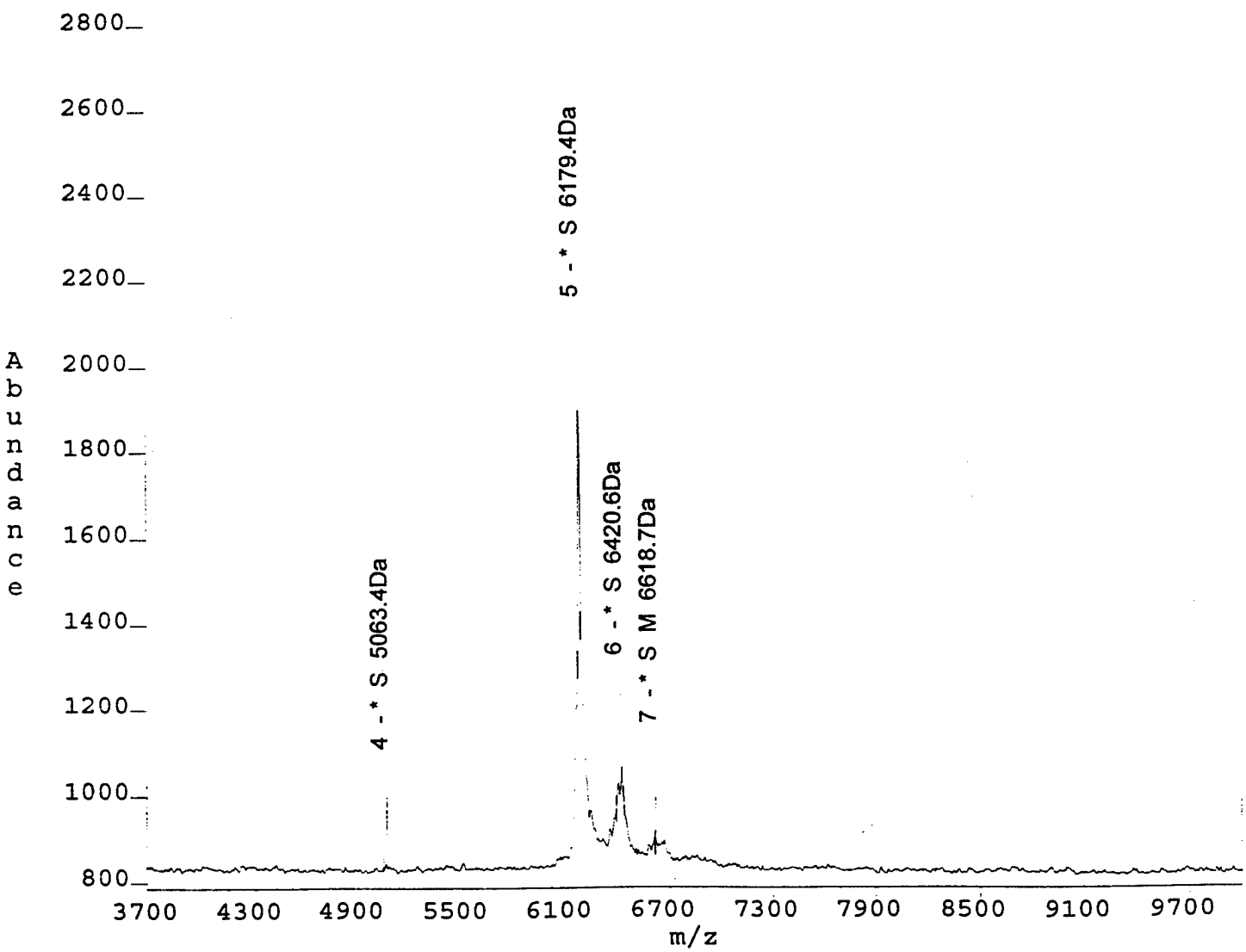
4/14/98

Collected	Fri Apr 17 11:40:46 1998
Processed	Fri Apr 17 11:42:08 1998
Printed	Fri Apr 17 11:42:20 1998

Sequence			
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET		
Collection Mode	Auto Multi Shots (S/N 77.4) (50 of 69) Mesa 5 [25-25]		
Laser Energy	1.05 (0.17) uJ	Vacuum	9.47e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Figure 1C



Sign: _____ Date: / /

Figure 1c (cont'd

NEW DATA* [A.03.00 , #5095]

Sample Name	EGF/SANPAH 1:5
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

4/14/98

Collected	Fri Apr 17 11:40:46 1998
Processed	Fri Apr 17 11:42:08 1998
Printed	Fri Apr 17 11:42:20 1998

Sequence	
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET

Collection Mode	Auto Multi Shots (S/N 77.4) (50 of 69) Mesa 5 [25-25]		
Laser Energy	1.05 (0.17) uJ	Vacuum	9.47e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Peak			Height	Area	MW	delMW	%err	Name
1	*	S	893	726	1113.2			(page 1 of 1)
2	*	S	942	790	1200.5	87.2		
3	*	S	891	615	1224.3	23.9		
4	*	S	847	510	5063.4	3839.1		
5	*	S	1900	20074	6179.4	1116.0		
6	*	S	1069	5906	6420.6	241.2		
7	*	S M	906	63	6618.7	198.1		
8	*	S	891	2289	12363.1	5744.5		
9		S	856	618	12865.1	502.0		

*=Gauss, (D)eflected, (C)alibrant/(S)ample, M=Manual, P#=Polymer, ? = changed.

Sign: _____ Date: / /

NEW DATA* [A.03.00 , #5132]

Sample Name	EGF/SANPAH 1:7.5
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

4/14/98

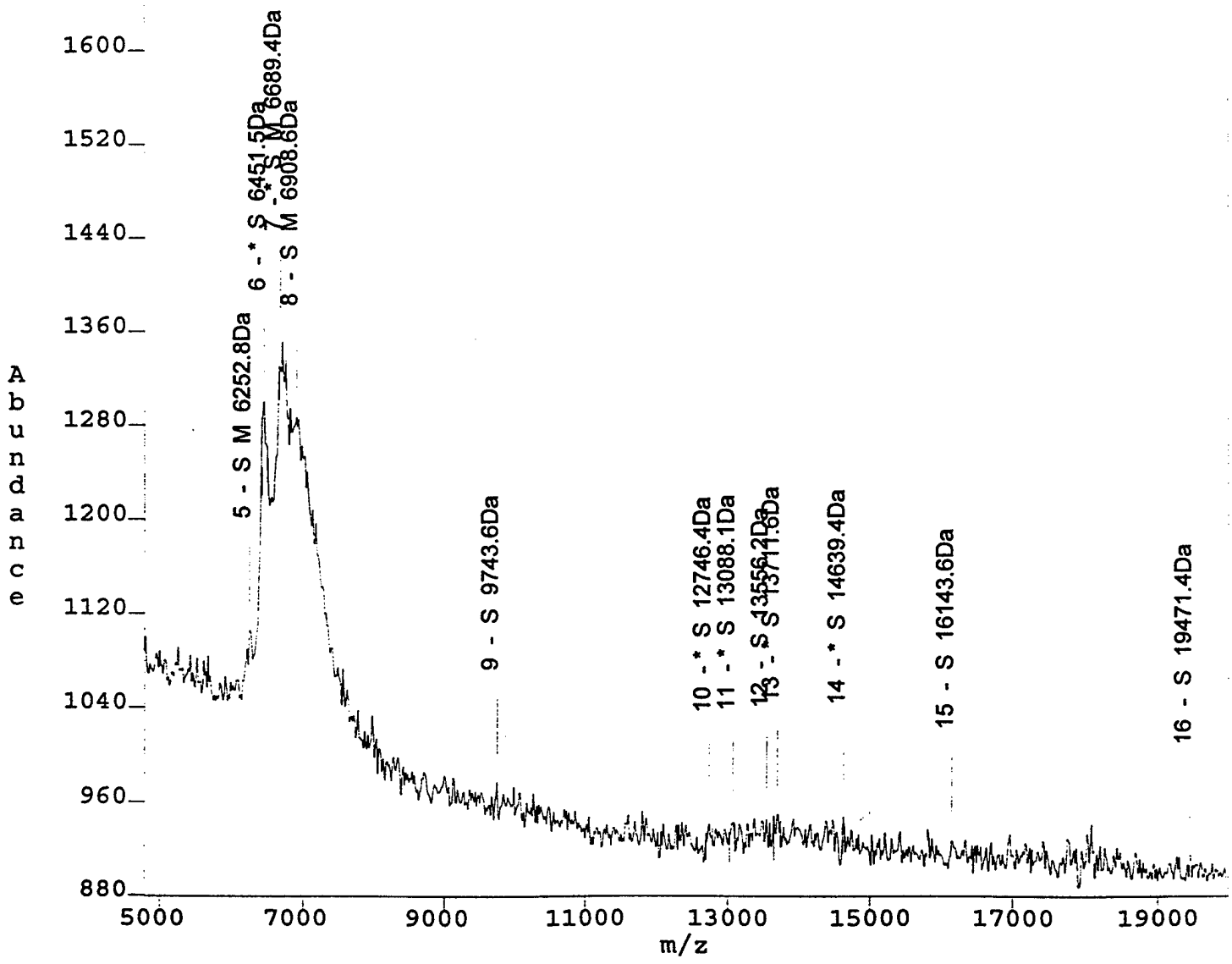
Collected	Mon Apr 20 12:43:25 1998
Processed	Mon Apr 20 12:52:40 1998
Printed	Mon Apr 20 12:54:33 1998

Sequence	
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET*

Collection Mode	Single Shots (55 of 263) Mesa 6 [14-117]		
Laser Energy	3.06 (0.52) uJ	Vacuum	7.03e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Figure 1D



Sign: _____ Date: / /

Figure 1D (cont'd)

NEW DATA* [A.03.00 , #5132]

Sample Name	EGF/SANPAH 1:7.5
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

4/14/98

Collected	Mon Apr 20 12:43:25 1998
Processed	Mon Apr 20 12:52:40 1998
Printed	Mon Apr 20 12:54:33 1998

Sequence			
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET*		
Collection Mode	Single Shots (55 of 263) Mesa 6 [14-117]		
Laser Energy	3.06 (0.52) uJ	Vacuum	7.03e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Peak			Height	Area	MW	delMW	%err	Name	(page 1 of 1)
1	*	S	3177	8684	1159.0				
2	*	S	3442	14390	1182.1	23.1			
3	*	S	2480	4930	1404.9	222.8			
4	*	S	3230	12002	1428.1	23.2			
5		S M	1106	65	6252.8	4824.7			
6	*	S	1300	5474	6451.5	198.7			
7	*	S M	1351	2119	6689.4	238.0			
8		S M	1287	-64	6908.6	219.2			
9		S	977	553	9743.6	2835.0			
10	*	S	941	665	12746.4	3002.8			
11	*	S	943	702	13088.1	341.7			
12		S	945	543	13556.2	468.1			
13	*	S	950	934	13711.6	155.4			
14	*	S	947	509	14639.4	927.7			
15		S	927	545	16143.6	1504.2			
16		S	915	519	19471.4	3327.8			

*=Gauss, (D)eflected, (C)alibrant/(S)ample, M=Manual, P#=Polymer, ? = changed.

Sign: _____ Date: / /

Figure 1E

NEW DATA* [A.03.00 , #5119]

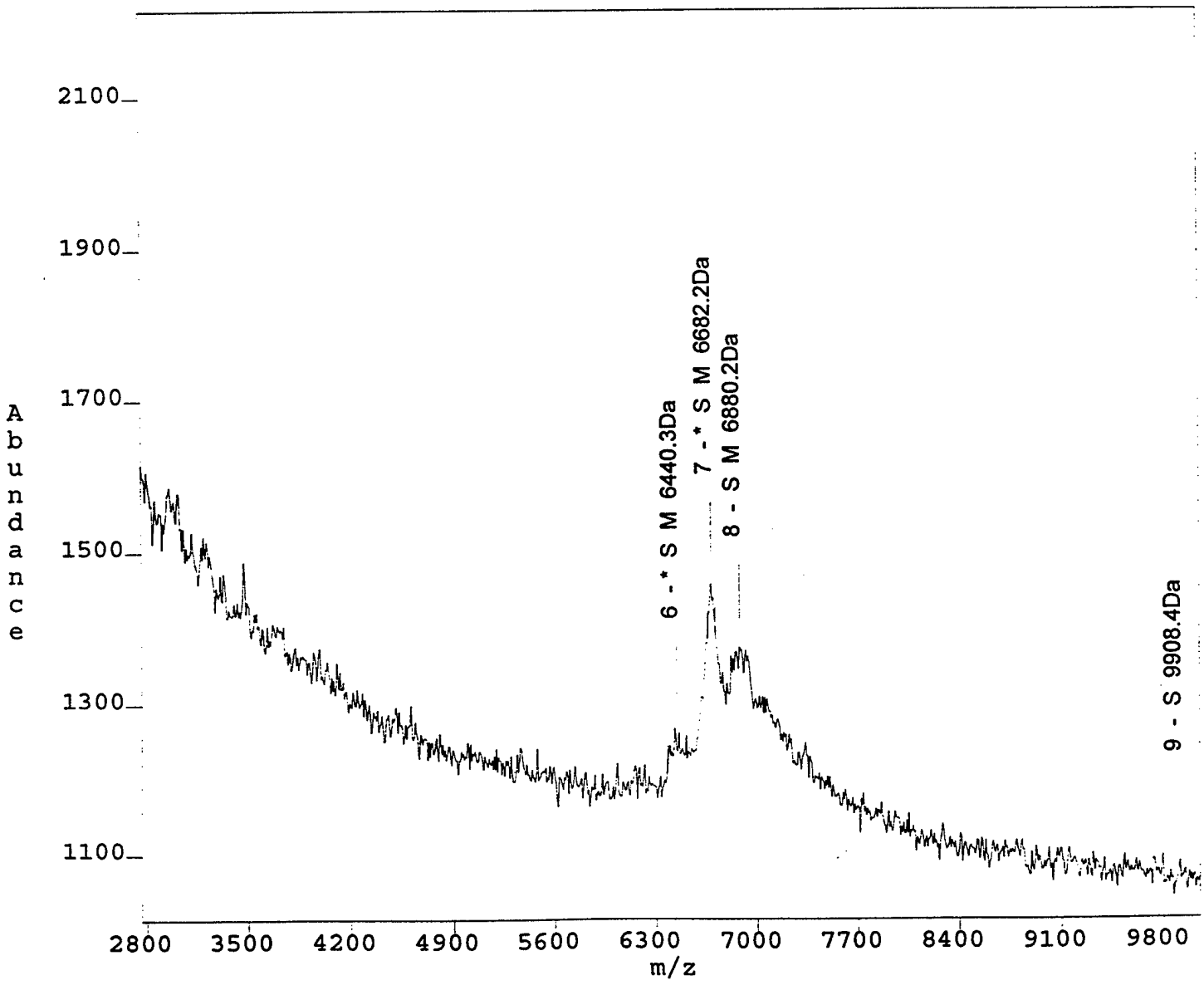
Sample Name	EGF/SANPAH 1:~~7.5~~ 10
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

4/14/98

Collected	Sat Apr 18 14:52:38 1998
Processed	Sat Apr 18 15:05:43 1998
Printed	Sat Apr 18 15:06:10 1998

Sequence			
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET		
Collection Mode	Single Shots (57 of 106) Mesa 7 [41-98]		
Laser Energy	2.95 (0.61) uJ	Vacuum	4.10e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]



Sign: _____ Date: / /

NEW DATA* [A.03.00 , #5119]

Figure 1 E (cont'd)

Sample Name	EGF/SANPAH 1:~~7.5~~ 10
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

4/14/98

Collected	Sat Apr 18 14:52:38 1998
Processed	Sat Apr 18 15:05:43 1998
Printed	Sat Apr 18 15:06:10 1998

Sequence	
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET

Collection Mode	Single Shots (57 of 106) Mesa 7 [41-98]		
Laser Energy	2.95 (0.61) uJ	Vacuum	4.10e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Peak			Height	Area	MW	delMW	%err	Name
1	* S		3034	8516	1158.0			(page 1 of 1)
2	* S		2930	6516	1180.8	22.8		
3	* S		3521	14052	1202.7	21.9		
4	* S		3140	12575	1425.3	222.7		
5	* S		3177	10810	1447.8	22.5		
6	* S	M	1268	2433	6440.3	4992.4		– EGF/SAN (1:1)
7	* S	M	1458	2446	6682.2	242.0		– EGF/SAN (1:2)
8	S	M	1375	1421	6880.2	198.0		– EGF/SAN (1:3)?
9	S		1089	509	9908.4	3028.2		
10	S		1085	565	10795.9	887.4		
11	S		1062	937	12608.1	1812.2		
12	* S		1059	658	13273.9	665.8		
13	S		1064	765	13384.4	110.5		
14	S		1064	772	14319.9	935.5		
15	* S		1062	867	15132.0	812.1		
16	* S		1047	517	15407.7	275.7		
17	* S		1026	582	18507.7	3100.0		
18	S		1033	886	18622.7	115.0		
19	S		1026	544	19003.8	381.2		

*=Gauss, (D)eflected, (C)alibrant/(S)ample, M=Manual, P#=Polymer, ? = changed.

Sign: _____ Date: / /

NEW DATA* [A.03.00 , #5108]

Sample Name	EGF/Genistein 1:10 not pre-photolyzed
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

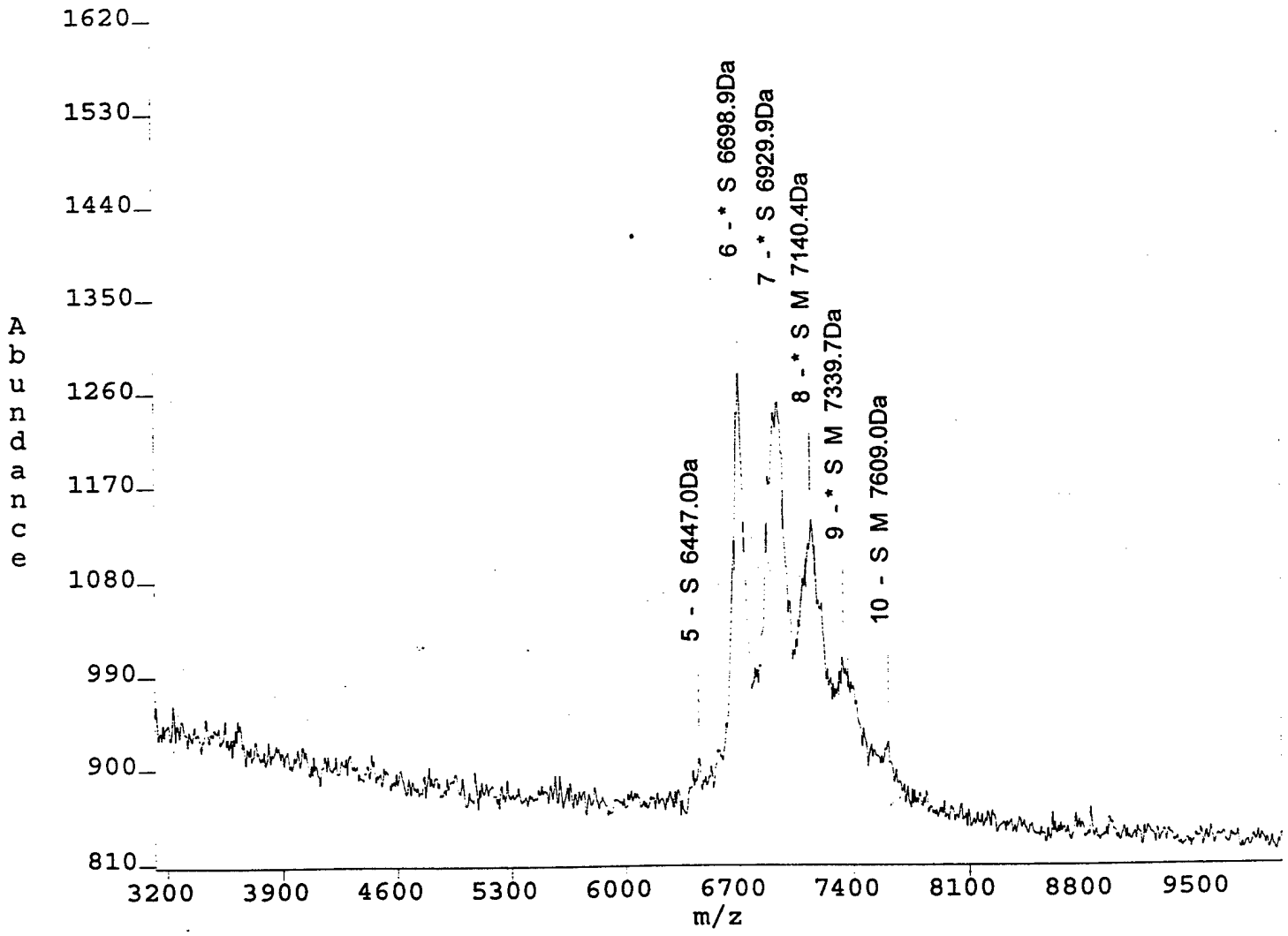
Figure 1F

4/15/98

Collected	Fri Apr 17 13:33:31 1998
Processed	Fri Apr 17 13:35:31 1998
Printed	Fri Apr 17 13:35:41 1998

Sequence			
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET		
Collection Mode	Auto Multi Shots (S/N 31.1) (50 of 113) Mesa 10 [57-59]		
Laser Energy	1.76 (0.53) uJ	Vacuum	6.17e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]



Sign: _____ Date: / /

Figure 1F (cont'd)

NEW DATA* [A.03.00 , #5108]

Sample Name	EGF/Genistein 1:10
Preparation	PBS
Matrix	Sinnapinic Acid
User Name	L. Ronken
Department Name	Biotherapy
Application	

4/15/98

Collected	Fri Apr 17 13:33:31 1998
Processed	Fri Apr 17 13:35:31 1998
Printed	Fri Apr 17 13:35:41 1998

Sequence			
Method	C:\HPTOFOLD\METHOD\PEP-NEG.MET		
Collection Mode	Auto Multi Shots (S/N 31.1) (50 of 113) Mesa 10 [57-59]		
Laser Energy	1.76 (0.53) uJ	Vacuum	6.17e-007 torr
Mass Range	20000 Da	Ion Optics	28.0/7.0 kV
Mass Filter	350 Da	Detector	-4.75 kV
Data Interval	5.0 nsec	Digitizer	1000 mVFS
Polarity	Negative	Filter	None

A2 5.1885630 A1 -0.4177490 A0 0.0084090 res 16.1913010
Calibration - Program Calculated (2-Parameter)
Calibration Date Fri Nov 04 15:09:44 1994
Calibrator Christopher M. Adams
Calib Data File C:\HPTOF\DATA\PEPNEG8.TOF [#2091]

Peak			Height	Area	MW	delMW	%err	Name
1	*	S	1491	3756	1075.8			
2	*	S	1771	7956	1119.5	43.7		
3	*	S	1387	6008	1208.1	88.6		
4	*	S	1283	1816	1454.3	246.2		
5		S	910	992	6447.0	4992.7		EGF/SAN(1:1)
6	*	S	1278	13601	6698.9	251.9		EGF/SAN/Gen or EGF/SAN (1:2)
7	*	S	1250	12509	6929.9	231.0		
8	*	S	M 1137	2790	7140.4	210.5		
9	*	S	M 1006	569	7339.7	199.3		
10		S	M 925	143	7609.0	269.3		

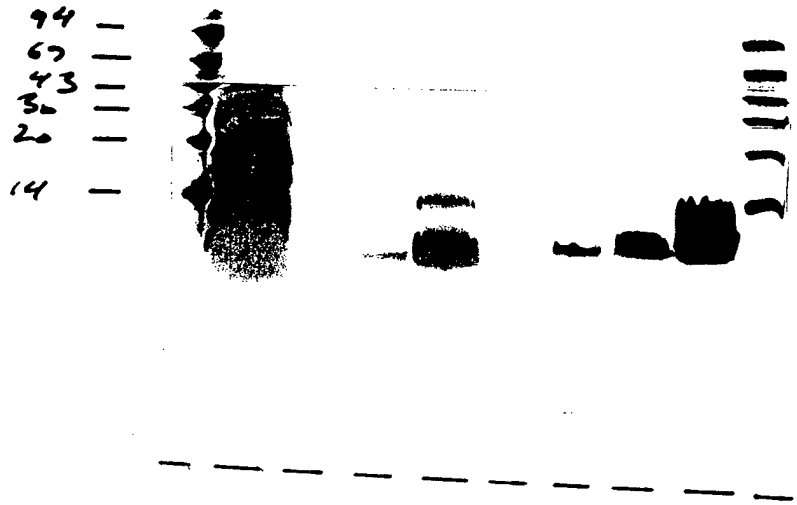
(page 1 of 1)

*=Gauss, (D)eflected, (C)alibrant/(S)ample, M=Manual, P#=Polymer, ? = changed.

Sign: _____ Date: / /

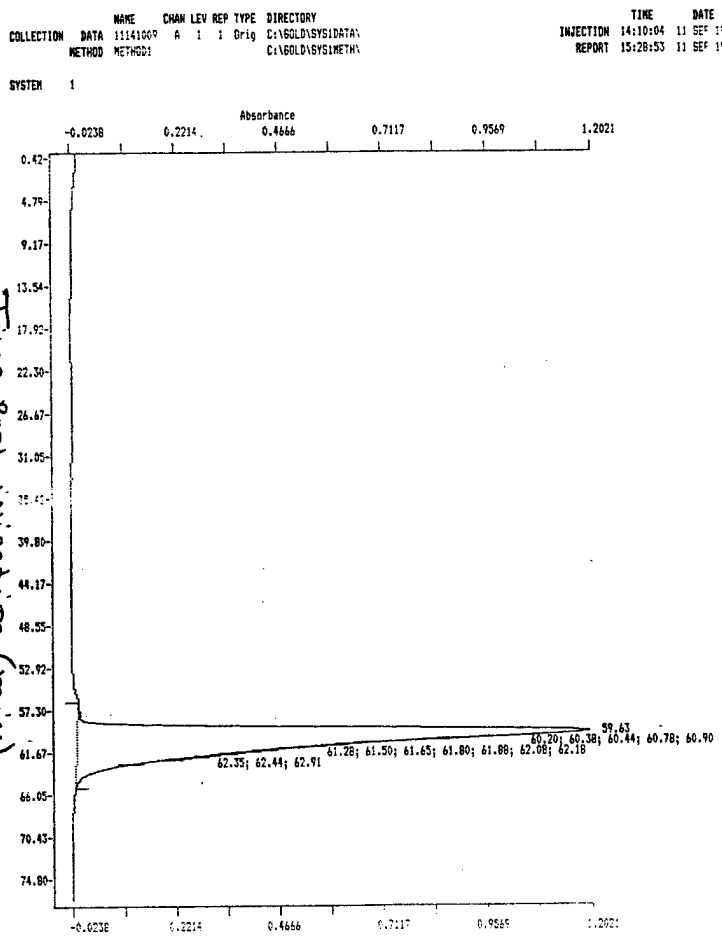
Tricine Gels
5-13-99
EGF & EGF/GEN

Figure 2



EGF

Figure 3A



EGF control for MTT (Fig. 4)

Figure 3A (cont'd)

bt20 EGF: Page: 1

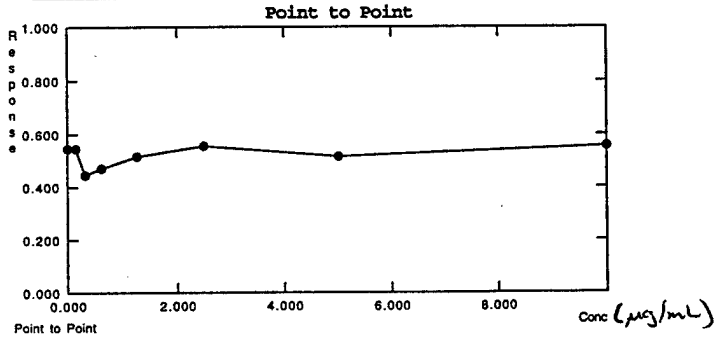
Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:
Protocol: DEF-10
Date: 07/24/98, 16:07:14
Segment: 1: A1-H12
Operator:

Comment:
Masked Wells:
Transformations:
1 : @-#, #=.1153333333
2 : @
3 : @
4 : @
Transf. Resp.:
Transf. Unit.:
Shake: Med, 10sec

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.013	0.543	0.223	0.543	0.540	0.525	0.518	0.545	0.535	0.444	0.484	0.008
B	-.002	0.538	0.391	0.507	0.487	0.448	0.478	0.425	0.450	0.444	0.477	-.007
C	-.011	0.562	0.456	0.578	0.487	0.496	0.498	0.485	0.453	0.451	0.438	-.005
D	# -.012	0.000 0.565	10.00 0.523	5.000 0.496	2.500 0.523	1.250 0.523	0.625 0.438	0.312 0.454	0.156 0.628	0.481	0.000 0.463	# -.012
E	# -.007	0.000 0.629	10.00 0.613	5.000 0.566	2.500 0.631	1.250 0.527	0.625 0.537	0.312 0.431	0.156 0.492	0.486	0.000 0.463	# 0.004
F	# 0.003	0.000 0.640	10.00 0.527	5.000 0.489	2.500 0.508	1.250 0.496	0.625 0.438	0.312 0.450	0.156 0.511	0.465	0.000 0.497	# 0.010
G	-.077	-.077	-.077	-.077	-.077	-.077	-.077	-.077	-.077	-.076	-.077	-.076
H	-.077	-.077	-.077	-.077	-.077	-.078	-.077	-.077	-.078	-.075	-.077	-.073

Point to Point



Point to Point

EGF control for MTT (Fig. 4)

Figure 3 A (cont'd)

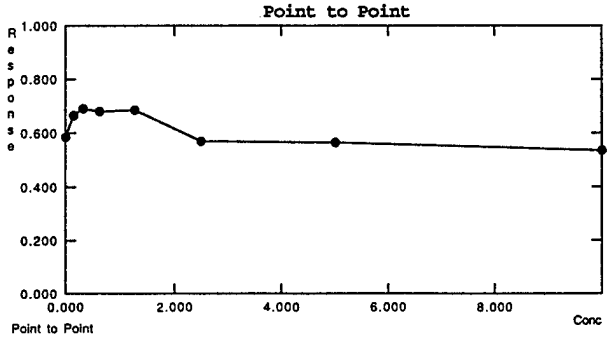
mdamb231 egf: Page: 1

Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:
Protocol: DEF-10
Date: 07/24/98, 16:40:33
Segment: 1: A1-H12
Operator:
Comment:
Masked Wells:
Transformations:
1 : @-#, #=.08088888889
2 : @
3 : @
4 : @
Transf. Resp.:
Transf. Unit.:
Shake: Med, 10sec

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	-.002	0.616	0.520	0.611	0.631	0.625	0.764	0.724	0.653	0.611	0.552	0.002
B	-.004	0.615	0.494	0.575	0.611	0.633	0.690	0.656	0.604	0.648	0.598	0.002
C	-.003	0.618	0.495	0.513	0.628	0.691	0.683	0.649	0.704	0.613	0.634	0.001
D	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.001	0.510	0.535	0.515	0.562	0.602	0.632	0.618	0.679	0.631	0.623	-.001
E	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.001	0.581	0.555	0.570	0.620	0.728	0.654	0.724	0.693	0.622	0.643	0.002
F	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.002	0.522	0.519	0.503	0.521	0.725	0.757	0.732	0.619	0.721	0.623	0.001
G	-.043	-.043	-.043	-.043	-.043	-.043	-.043	-.042	-.042	-.043	-.042	-.044
H	-.042	-.043	-.041	-.043	-.042	-.044	-.042	-.041	-.043	-.040	-.043	-.042

Point to Point



Point to Point

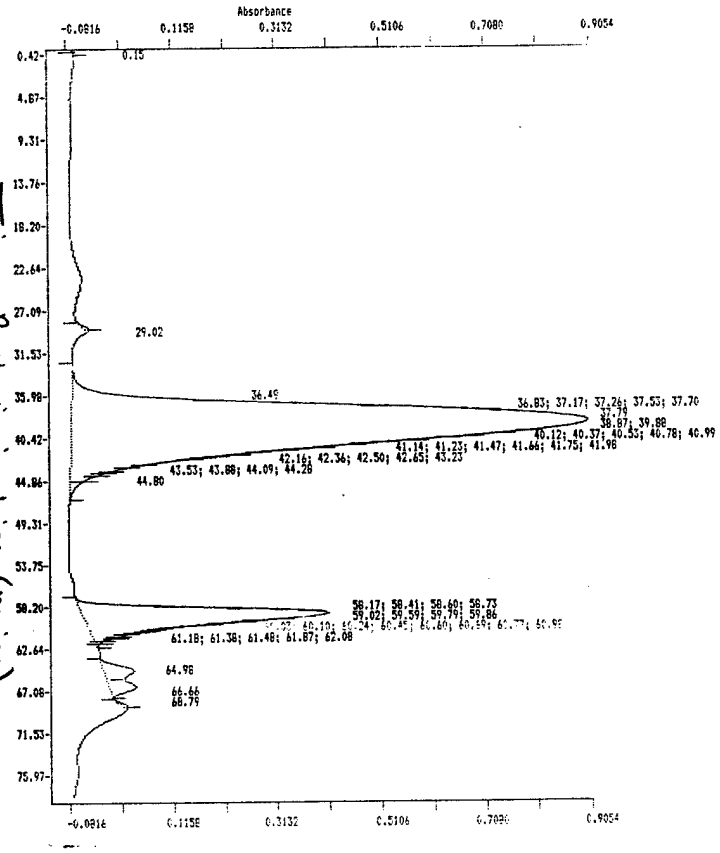
EGF/SAN-GeN
(1:4)(1:20) pp

Figure 3B

		NAME	CHAN	LEV	REP	TYPE	DIRECTORY
COLLECTION	DATA	05124648	A	1	1	Orig	C:\GOLD\SYS1DATA\
	METHOD	METHOD1					C:\GOLD\SYS1METH\

	TIME	DATE
INJECTION	12:46:43	5 JUL 199
REPORT	14:07:14	5 JUL 199

SYSTEM 1

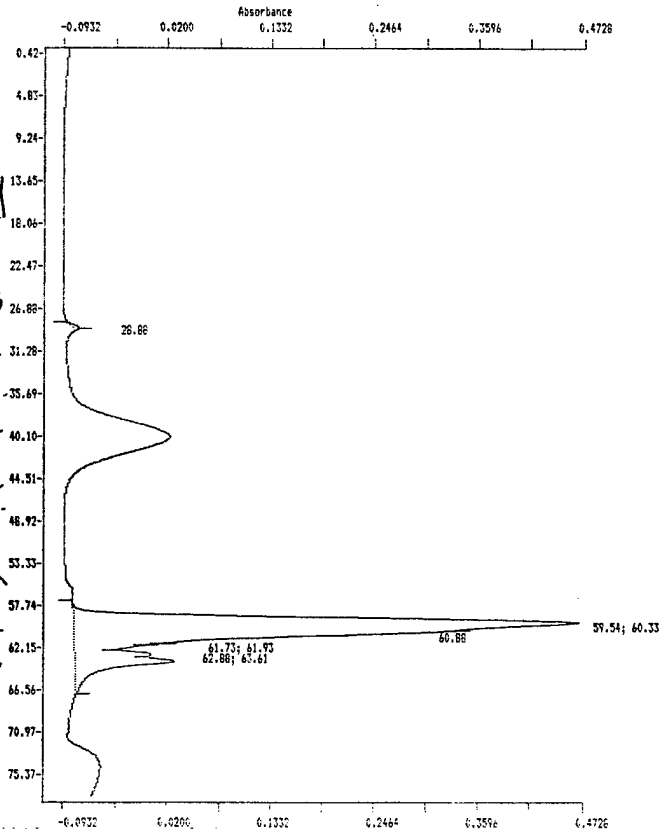


EGF/SAN-GeN
(1:2)(1:20) PP

Figure 3C

		NAME	CHAN	LEV	REP	TYPE	DIRECTORY		TIME	DATE
COLLECTION	DATA	07124811	A	1	1	Orig	C:\GOLD\SYSIDATA\	INJECTION	12:48:07	7 JUL 199
	METHOD	METHOD1					C:\GOLD\SYSIMETH\	REPORT	14:07:16	7 JUL 199

SYSTEM 1



EGF/ANB-NOS-Gen (1:10)(1:2.5) PP

Figure 4A

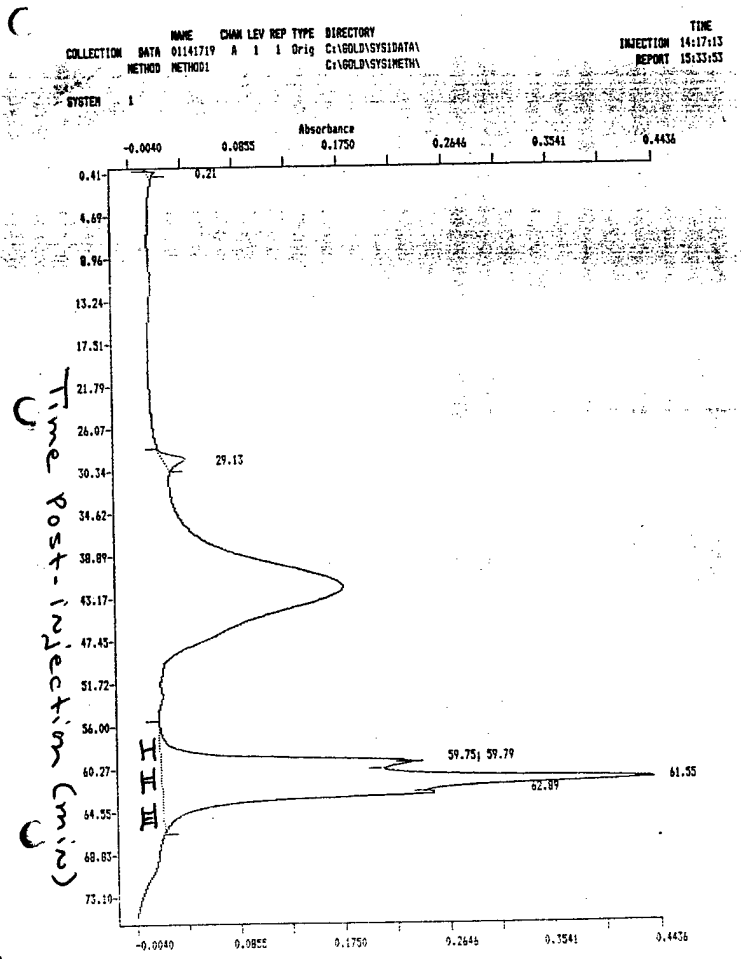


Fig. 4A (cont'd)

BT20 EGF/ANBNOSGEN 15'LW : (-HPLC) Page: 1

Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:

Comment:
Masked Wells:
Transformations:
1 : ⊘-#, #=.07183333333
2 : ⊘
3 : ⊘
4 : ⊘
Shake: Med, 10sec

Protocol: ABC-10
Date: 09/08/98, 17:29:42
Segment: 1: A1-H12
Operator:

(1:10)(1:2.5)
120 hour incubation
%

Transf. Resp.:
Transf. Unit.:

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.002	0.303	0.074	0.113	0.233	0.356	0.248	0.305	0.318	0.288	0.261	0.008
B	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.006	0.300	0.071	0.069	0.221	0.284	0.257	0.293	0.263	0.281	0.241	0.004
C	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.006	0.317	0.059	0.054	0.231	0.277	0.284	0.285	0.298	0.293	0.235	0.001
D	-.006	0.305	0.081	0.295	0.310	0.329	0.283	0.271	0.293	0.312	0.295	0.004
E	-.004	0.298	0.092	0.283	0.280	0.330	0.322	0.313	0.317	0.256	0.253	0.006
F	-.003	0.294	0.093	0.301	0.246	0.311	0.320	0.320	0.320	0.289	0.272	0.007
G	-.034	-.034	-.035	-.035	-.034	-.035	-.035	-.034	-.034	-.035	-.033	-.035
H	-.036	-.036	-.033	-.035	-.035	-.035	-.034	-.035	-.034	-.032	-.035	-.035

Point to Point

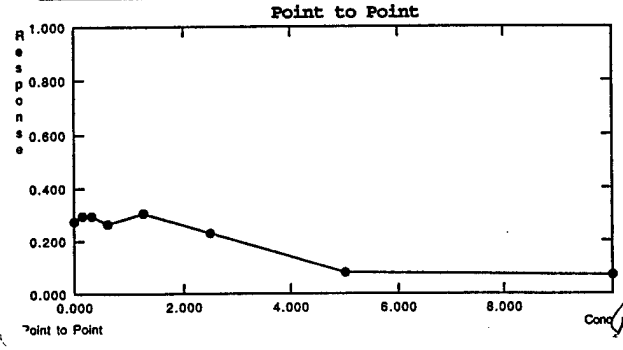


Fig 4A (cont'd)

BT20 EGF/ANBNOSGEN 15'LWHPLCHI: Page: 1

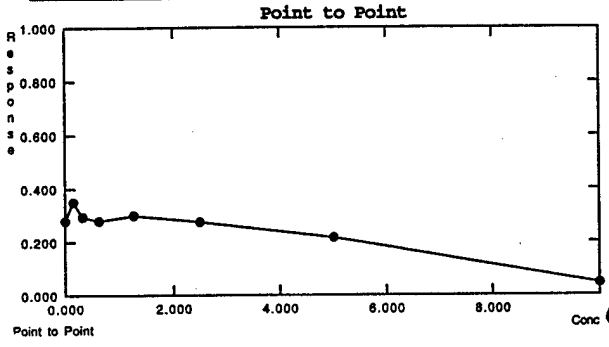
Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:
Comment:
Masked Wells:
Transformations:
1 : @-#, #=.081
2 : @
3 : @
4 : @
Shake: Med, 10sec

Protocol: DEF-10
Date: 09/08/98, 17:32:08
Segment: 1: A1-H12
Operator:
(1:10) (1:2.5)
+72 hour incubation
Transf. Resp.: 96
Transf. Unit.:

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	-.009	0.278	0.281	0.273	0.276	0.383	0.229	0.245	0.279	0.225	0.218	-.003
B	-.011	0.336	0.244	0.309	0.297	0.299	0.285	0.289	0.316	0.220	0.253	-.009
C	-.012	0.250	0.238	0.281	0.305	0.261	0.290	0.263	0.349	0.282	0.285	-.007
D	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.015	0.281	0.040	0.196	0.234	0.256	0.268	0.294	0.370	0.250	0.237	-.008
E	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.013	0.301	0.047	0.210	0.290	0.299	0.298	0.313	0.362	0.293	0.255	-.004
F	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.006	0.313	0.051	0.245	0.296	0.338	0.279	0.278	0.324	0.309	0.287	0.046
G	-.044	-.045	-.043	-.042	-.041	-.043	-.042	-.042	-.042	-.042	-.042	-.044
H	-.043	-.043	-.043	-.043	-.043	-.043	-.043	-.042	-.042	-.040	-.044	-.044

Point to Point



EGF/ANB-NOS-GeN
(1:10)(1:10) pp

Figure 4B

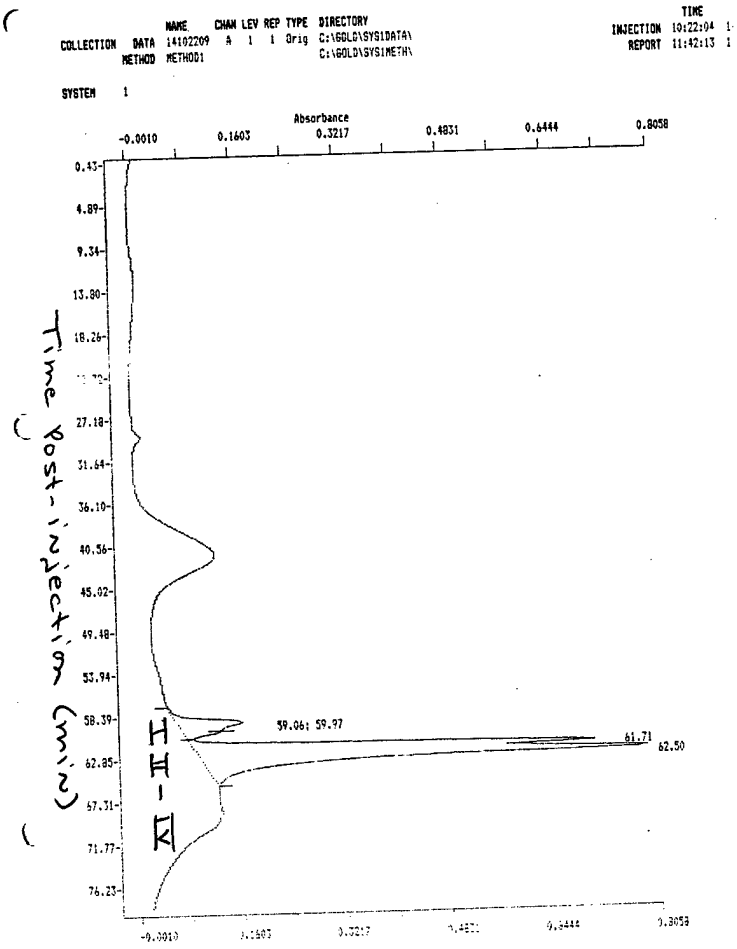


Fig 4B (cont'd)

BT20-EGFANBNOSGenHPLCIII7/14/98: Page: 1

Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:
Protocol: ABC-10
Date: 07/17/98, 17:17:02
Segment: 1: A1-H12
Operator:
48 hours

Comment:
Masked Wells:
Transformations:
1 : @-#, #=.1118333333
2 : @
3 : @
4 : @
Transf. Resp.:
Transf. Unit.:
Shake: Med, 10sec

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.009	0.333	0.120	0.109	0.123	0.136	0.115	0.125	0.133	0.351	0.389	0.003
B	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	0.009	0.368	0.124	0.145	0.157	0.135	0.108	0.119	0.119	0.358	0.372	0.001
C	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.001	0.378	0.114	0.143	0.142	0.133	0.104	0.116	0.140	0.429	0.383	-.004
D	-.024	0.368	0.126	0.135	0.145	0.143	0.113	0.114	0.150	0.363	0.401	-.001
E	0.137	0.358	0.148	0.145	0.152	0.152	0.124	0.120	0.148	0.355	0.411	0.002
	-.002	0.362	0.150	0.135	0.148	0.144	0.121	0.048	0.139	0.347	0.340	0.004
G	-.074	-.073	-.074	-.074	-.074	-.074	-.073	-.074	-.073	-.074	-.074	-.074
H	-.075	-.075	-.074	-.074	-.075	-.074	-.074	-.075	-.074	-.073	-.073	-.075

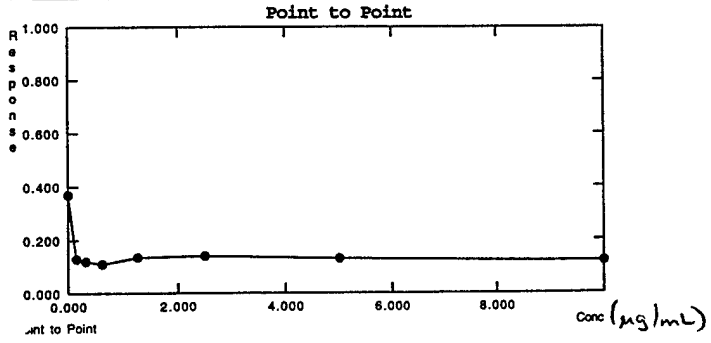


Fig 4B (cont'd)

MDAMB231-EGFANBNOSGenHPLCIII15': 7/17/98 Page: 1

Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:

Protocol: ABC-10
Date: 07/17/98, 17:36:40
Segment: 1: A1-H12
Operator:

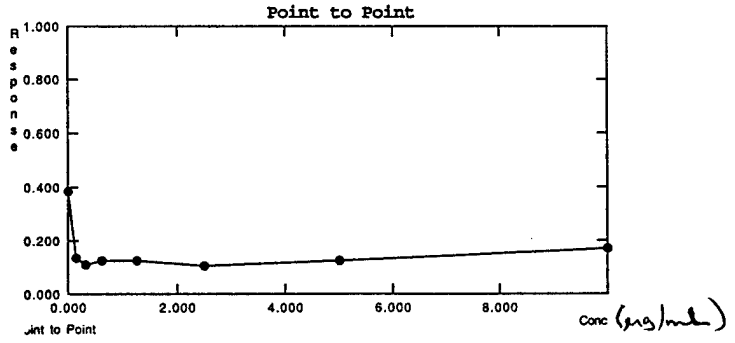
48 hours

Comment:
Masked Wells:
Transformations:
1 : ⊘-#, #=.0685
2 : ⊘
3 : ⊘
4 : ⊘
Shake: Med, 10sec

Transf. Resp.:
Transf. Unit.:

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	0.000	0.394	0.133	0.112	0.095	0.170	0.130	0.132	0.129	0.402	0.344	0.003
B	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.004	0.408	0.167	0.152	0.121	0.107	0.127	0.104	0.138	0.400	0.401	-.004
C	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	0.013	0.376	0.206	0.113	0.103	0.096	0.110	0.099	0.131	0.375	0.377	-.008
D	0.002	0.417	0.307	0.207	0.230	0.122	0.131	0.128	0.252	0.401	0.410	-.000
E	-.000	0.358	0.314	0.229	0.213	0.125	0.186	0.113	0.191	0.369	0.417	-.002
	-.006	0.396	0.333	0.207	0.222	0.140	0.206	0.131	0.204	0.401	0.405	-.008
G	-.030	-.029	-.030	-.029	-.030	-.029	-.028	-.029	-.030	-.027	-.030	-.031
H	-.031	-.031	-.030	-.029	-.030	-.031	-.030	-.030	-.030	-.027	-.030	-.030



EGF/ANB-NOS-GeN
(1:10)(1:20) not pp

Figure 4C

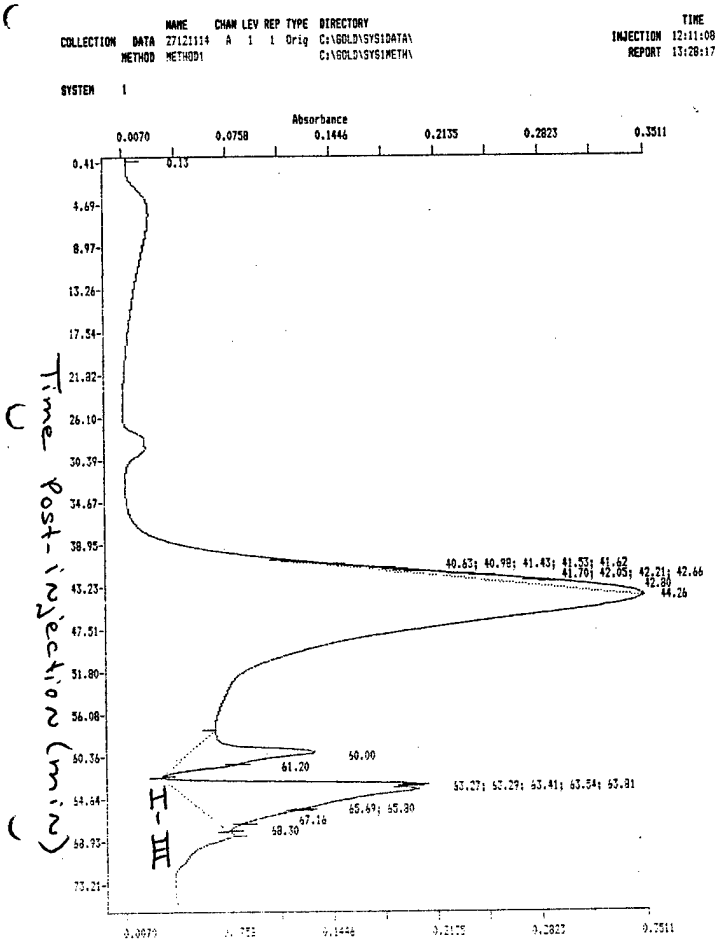


Figure 4c (cont'd)

BT20/EGFANBGen notpp1-20HPLCIII: Page: 1

Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:
Comment:
Masked Wells:
Transformations:
1 : @-#, #=.0945
2 : @
3 : @
4 : @
Shake: Med, 10sec

Protocol: DEF-10
Date: 08/01/98, 10:20:35
Segment: 1: A1-H12
Operator: (1:10)(1:20) 15' Lisa LW 7/27/98
Transf. Resp.: 72h. incubation
Transf. Unit.:

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	-.000	0.447	0.322	0.519	0.511	0.523	0.506	0.525	0.525	0.526	0.524	0.011
B	-.006	0.467	0.332	0.511	0.502	0.507	0.498	0.504	0.501	0.482	0.493	0.000
C	-.001	0.532	0.311	0.511	0.507	0.486	0.491	0.502	0.511	0.585	0.569	0.000
D	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.002	0.519	0.243	0.345	0.513	0.484	0.462	0.452	0.512	0.525	0.483	-.002
E	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	0.000	0.453	0.259	0.314	0.518	0.474	0.478	0.469	0.479	0.514	0.557	0.000
F	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	0.002	0.459	0.251	0.307	0.519	0.459	0.497	0.485	0.498	0.499	0.483	0.001
G	-.056	-.056	-.056	-.056	-.056	-.055	-.056	-.056	-.056	-.056	-.056	-.055
H	-.056	-.054	-.052	-.054	-.056	-.057	-.056	-.057	-.056	-.053	-.055	-.055

Point to Point

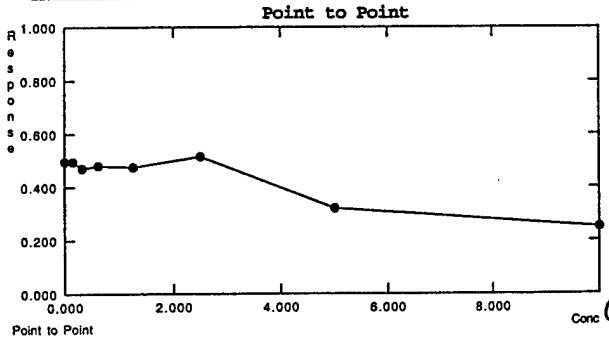


Figure 4c (cont'd)

MDAMB231EGFANBGen nopp HPLCIII: Page: 1

Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:
Comment:
Masked Wells: A3 C1 C11
Transformations:
1 : @-#, #=.073
2 : @
3 : @
4 : @
Shake: Med, 10sec

Protocol: DEF-10
Date: 08/01/98, 10:42:17
Segment: 1: A1-H12
Operator: (1:10)(1:20)
151 Lisa LWS not P/D
Transf. Resp.: 7/27/98
Transf. Unit.: 72h, incubation

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	-.001	0.726	X	0.704	0.745	0.745	0.752	0.708	0.791	0.761	0.784	-.001
B	-.003	0.773	0.618	0.719	0.769	0.777	0.789	0.740	0.799	0.749	0.776	-.002
C	X	0.782	0.752	0.714	0.820	0.808	0.794	0.765	0.851	0.780	X	-.002
D	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.002	0.748	0.624	0.663	0.748	0.766	0.763	0.790	0.806	0.756	0.767	0.001
E	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	-.003	0.757	0.474	0.615	0.709	0.710	0.748	0.787	0.798	0.719	0.779	0.002
F	#	0.000	10.00	5.000	2.500	1.250	0.625	0.312	0.156		0.000	#
	0.003	0.735	0.518	0.661	0.719	0.717	0.771	0.795	0.806	0.715	0.741	-.001
G	-.034	-.034	-.040	-.042	-.043	-.042	-.042	-.041	-.043	-.043	-.036	-.036
H	-.034	-.034	-.033	-.033	-.033	-.033	-.035	-.033	-.034	-.032	-.035	-.036

Point to Point

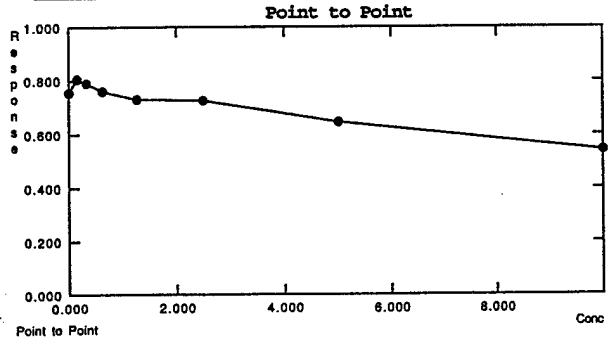


Fig 5

Date: 10/6/98 Gel #: EGF A2

PAGE 3½n %

Buffer System: Laemmli (Tricine) Other _____

Lane	Sample	Volume (ul)	
1	LMW STD	1-0	
2	LMW STD	1-0	
3	EGF/m*/Gen (1:10)(1:10) 1hr LW UV -HPLC 0.2ug	2.5	
4	" " " -HPLC 0.5ug	6.3	
5	EGF/m/Gen HPLC I 0.2ug	3.4	
6	" " " 0.5ug	8.5	
7	EGF/m/Gen HPLC I 0.2ug	10.0	
8	" " " 0.5ug	25.0	
9	EGF/m (1:10) 10/5 0.2ug	1.1	
10	" " " 0.5ug	2.9	
11			
12			
13			
14			
15			

NB-NOS

Run: _____ volts 50 mA 2-3 hours

Blot: NC PVDF

Transfer: _____ mA _____ min

1 Ab: _____
Incubation: _____ @ _____ C

2 Ab: _____
Incubation: _____ @ _____ C

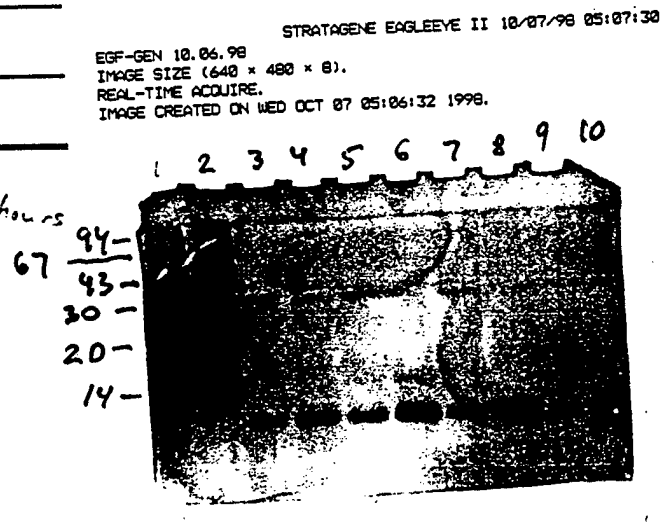
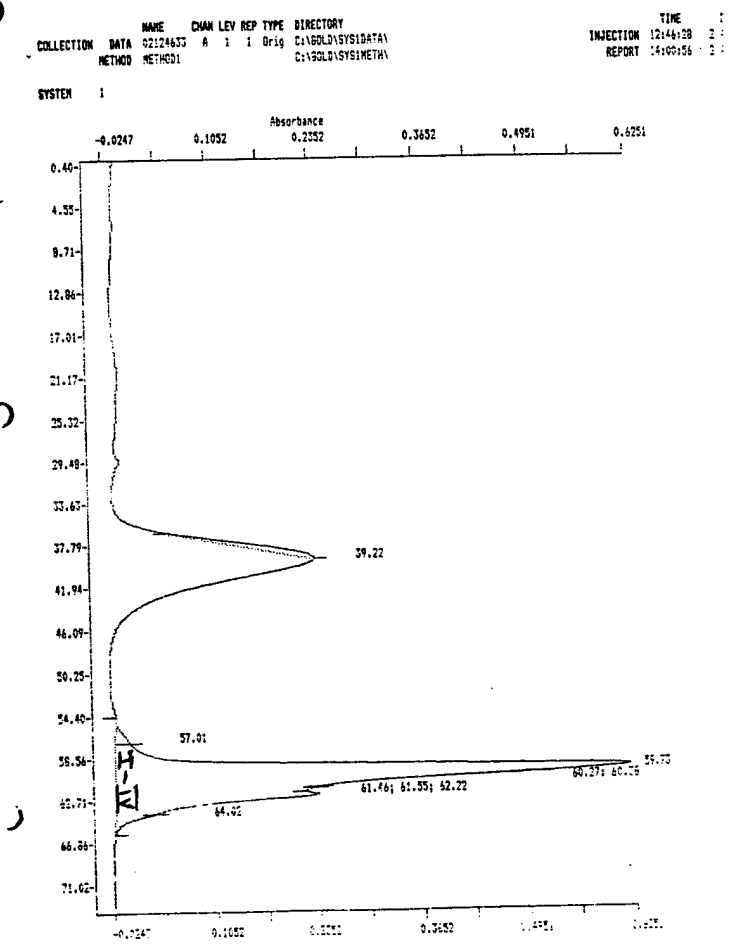


Fig 6A



EGF/41
(1:10) pH7.5

Fig 6A (cont'd)

T20 EGF/41 1-10 pH7.5 HPLCI: Page: 1

Mode: Endpoint 540 nm
Plate ID:
Conc.Unit:

Protocol: DEF-10
Date: 08/07/98, 16:20:45
Segment: 1: A1-H12
Operator:

Comment:
Masked Wells:
Transformations:
1 : @-#, #=.07916666667
2 : @
3 : @
4 : @
Shake: Med, 10sec

Transf. Resp.:
Transf. Unit.: 72hours

Plate & Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.003	0.467	0.480	0.505	0.462	0.425	0.410	0.415	0.445	0.450	0.499	-.006
B	0.001	0.515	0.569	0.531	0.507	0.508	0.447	0.549	0.467	0.393	0.485	-.007
C	0.001	0.557	0.588	0.538	0.483	0.538	0.565	0.545	0.429	0.442	0.458	-.009
D	# -.006	0.000 0.532	10.00 0.226	5.000 0.282	2.500 0.576	1.250 0.462	0.625 0.442	0.312 0.599	0.156 0.474	0.493	0.000 0.415	# -.010
E	# -.003	0.000 0.589	10.00 0.233	5.000 0.378	2.500 0.492	1.250 0.492	0.625 0.456	0.312 0.524	0.156 0.466	0.455	0.000 0.487	# -.004
F	# 0.023	0.000 0.602	10.00 0.185	5.000 0.335	2.500 0.488	1.250 0.469	0.625 0.665	0.312 0.856	0.156 0.505	0.498	0.000 0.460	# 0.001
G	-.041	-.040	-.040	-.040	-.040	-.041	-.040	-.040	-.040	-.040	-.040	-.042
H	-.039	-.039	-.041	-.040	-.040	-.040	-.042	-.041	-.041	-.040	-.041	-.042

Point to Point

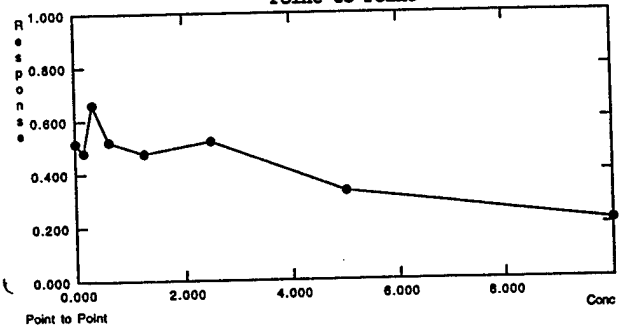


Fig 6B

EGF/24
(1:10) pH7.5

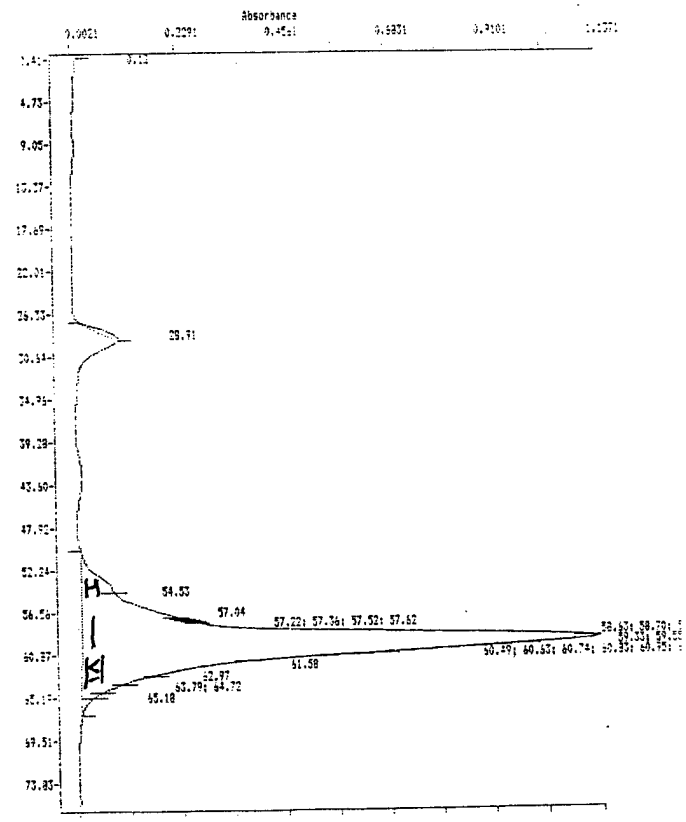


FIGURE 7a.

Effect of EGF/24 on Survival of Balb/c Mice

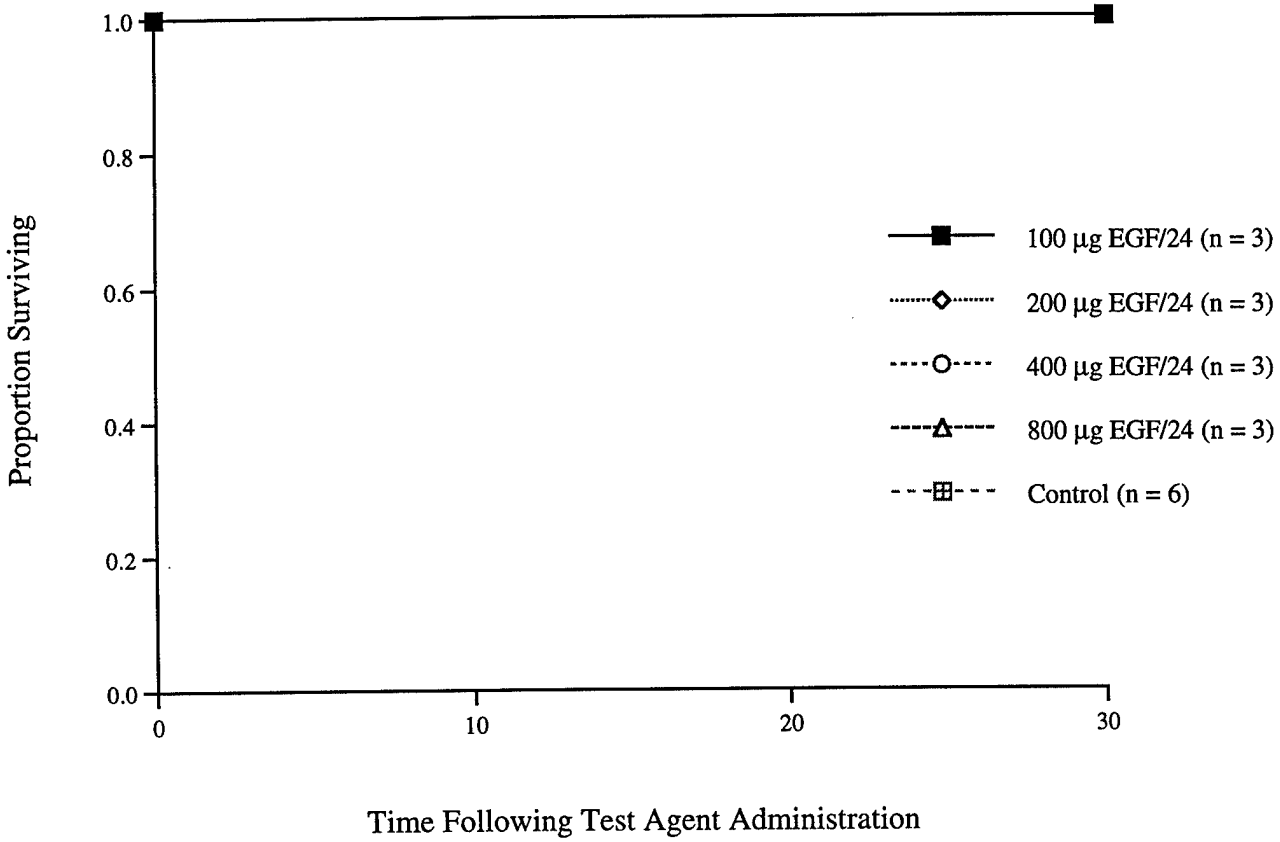


FIGURE 76.

Effect of EGF/41 on Survival of Balb/c Mice

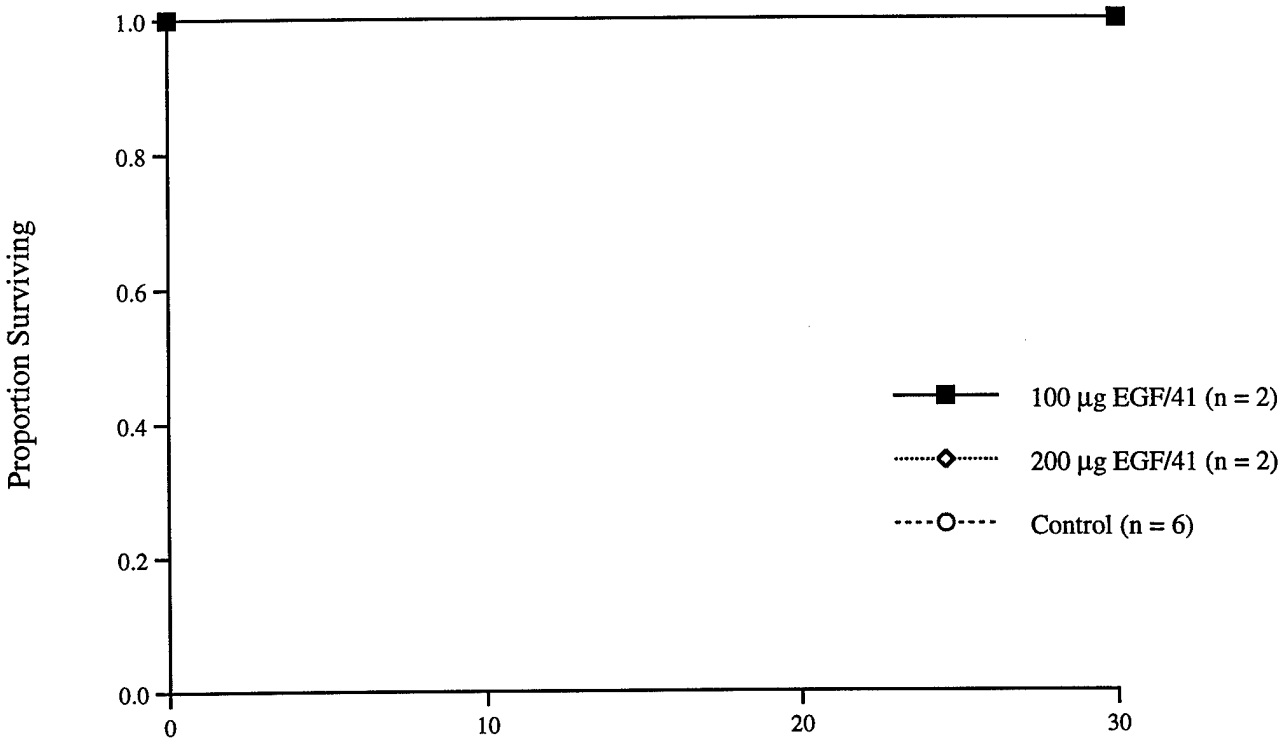


FIGURE 7c.

Effect of EGF/ANB-NOS-24 on Survival of Balb/c Mice

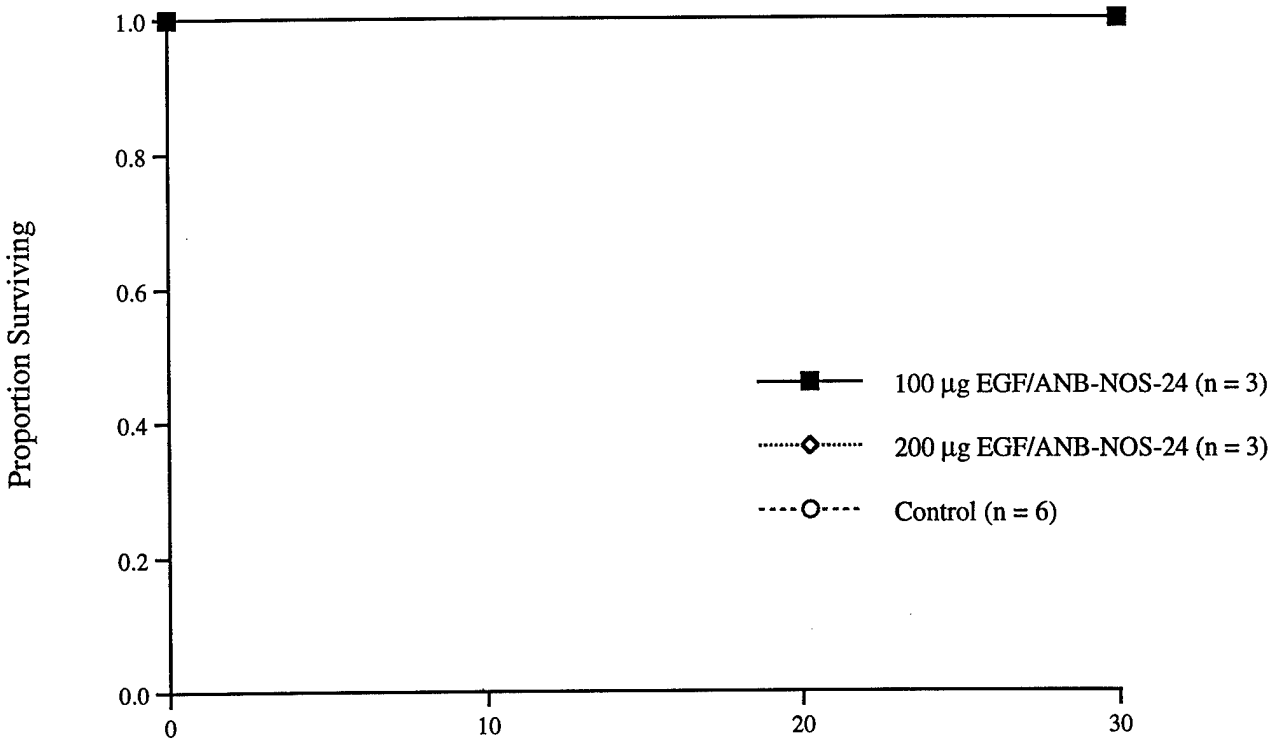
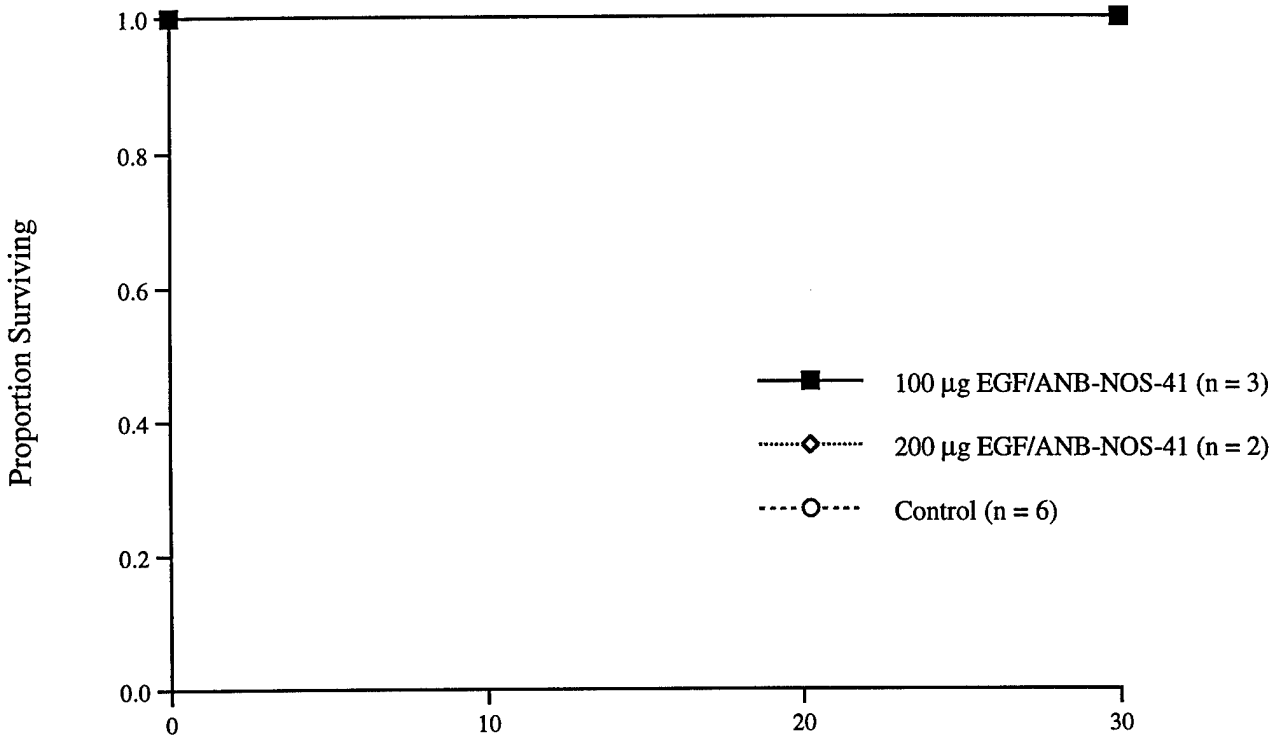


FIGURE 7d.

Effect of EGF/ANB-NOS-41 on Survival of Balb/c Mice



Time Following Test Agent Administration

FIGURE 7e.

Effect of EGF/ANB-NOS-Gen on Survival of Balb/c Mice

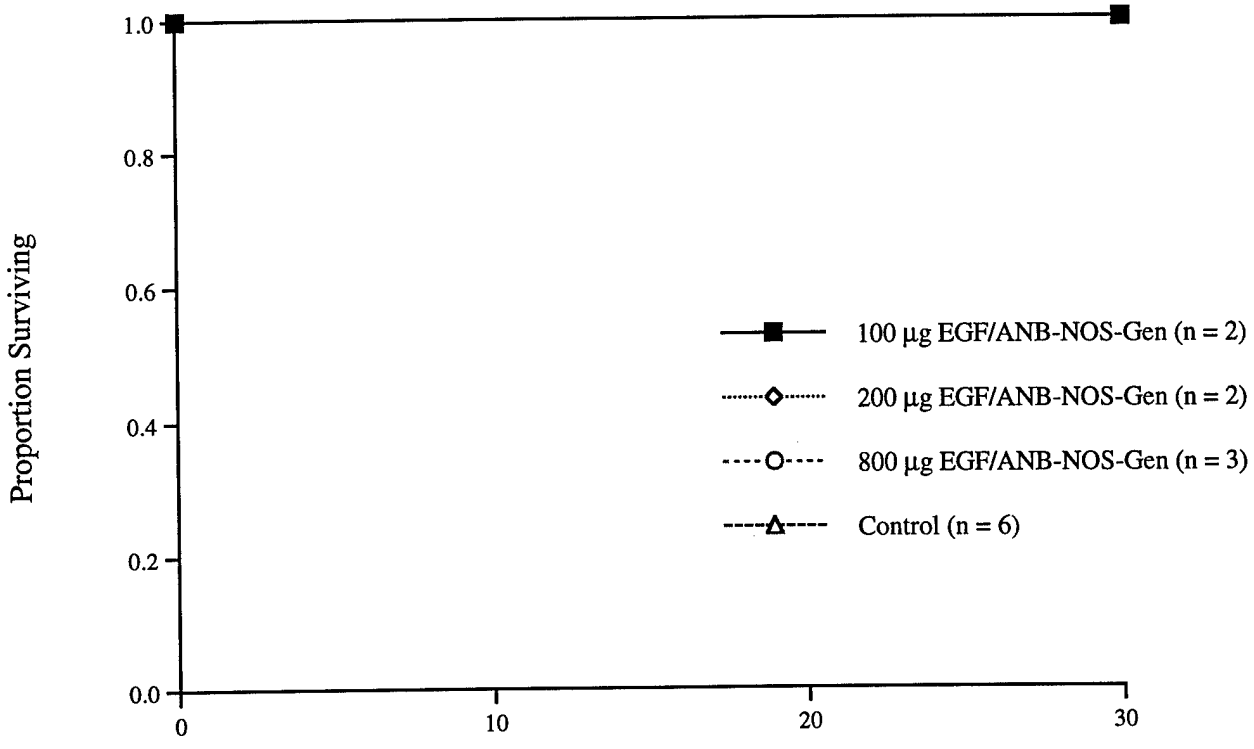


FIGURE 7f.

Effect of EGF/24 on Survival of Balb/c Mice

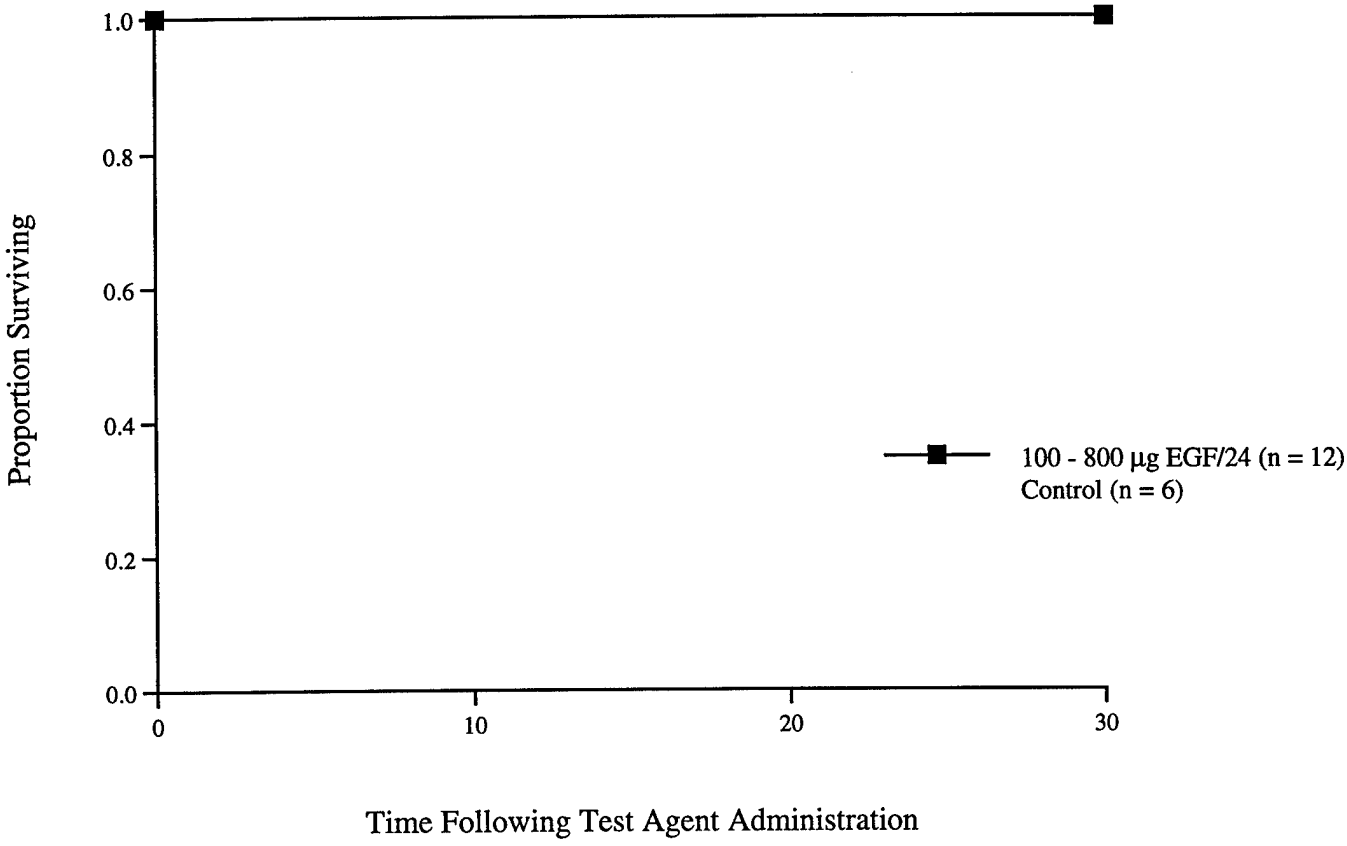


FIGURE 7g.

Effect of EGF/41 on Survival of Balb/c Mice

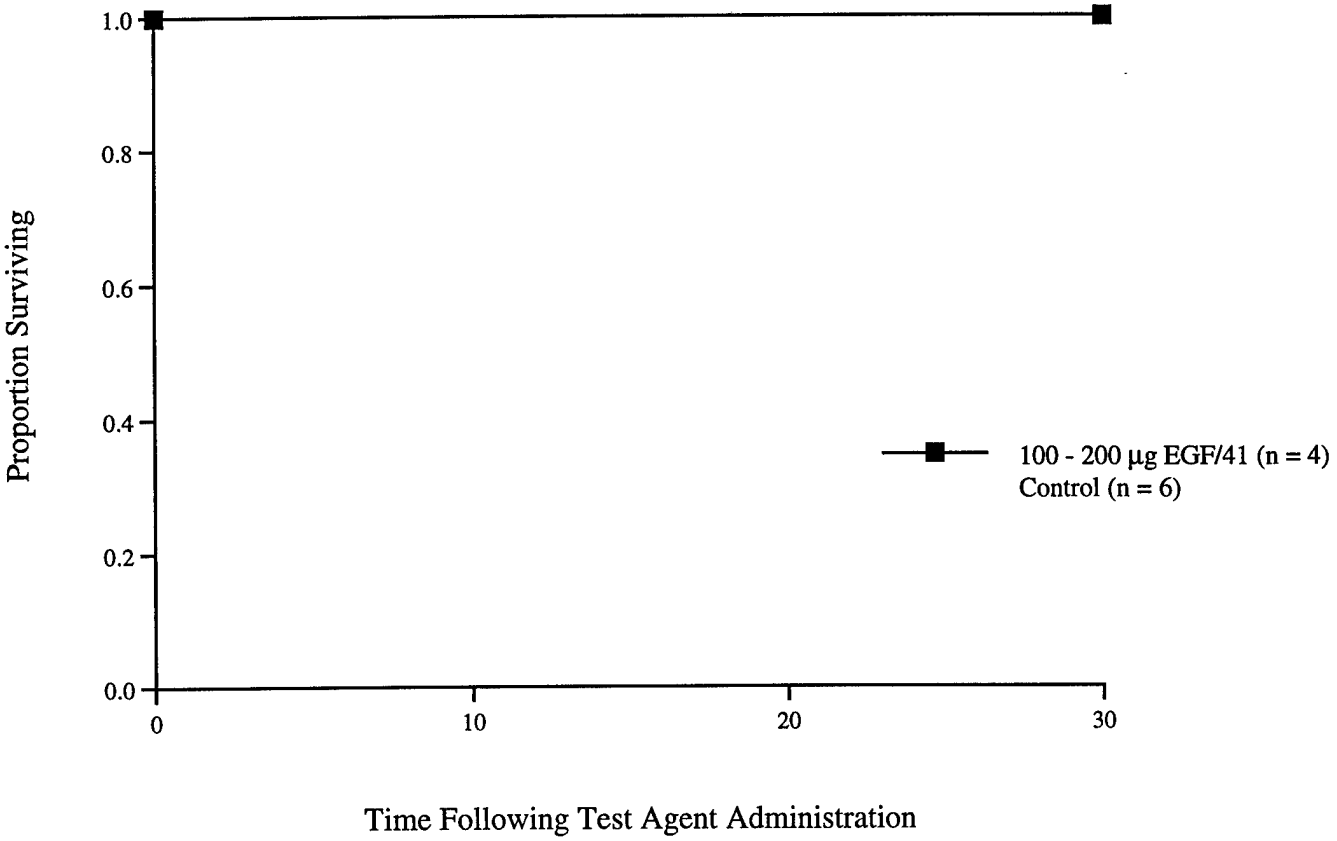


FIGURE 7h.

Effect of EGF/ANB-NOS-24 on Survival of Balb/c Mice

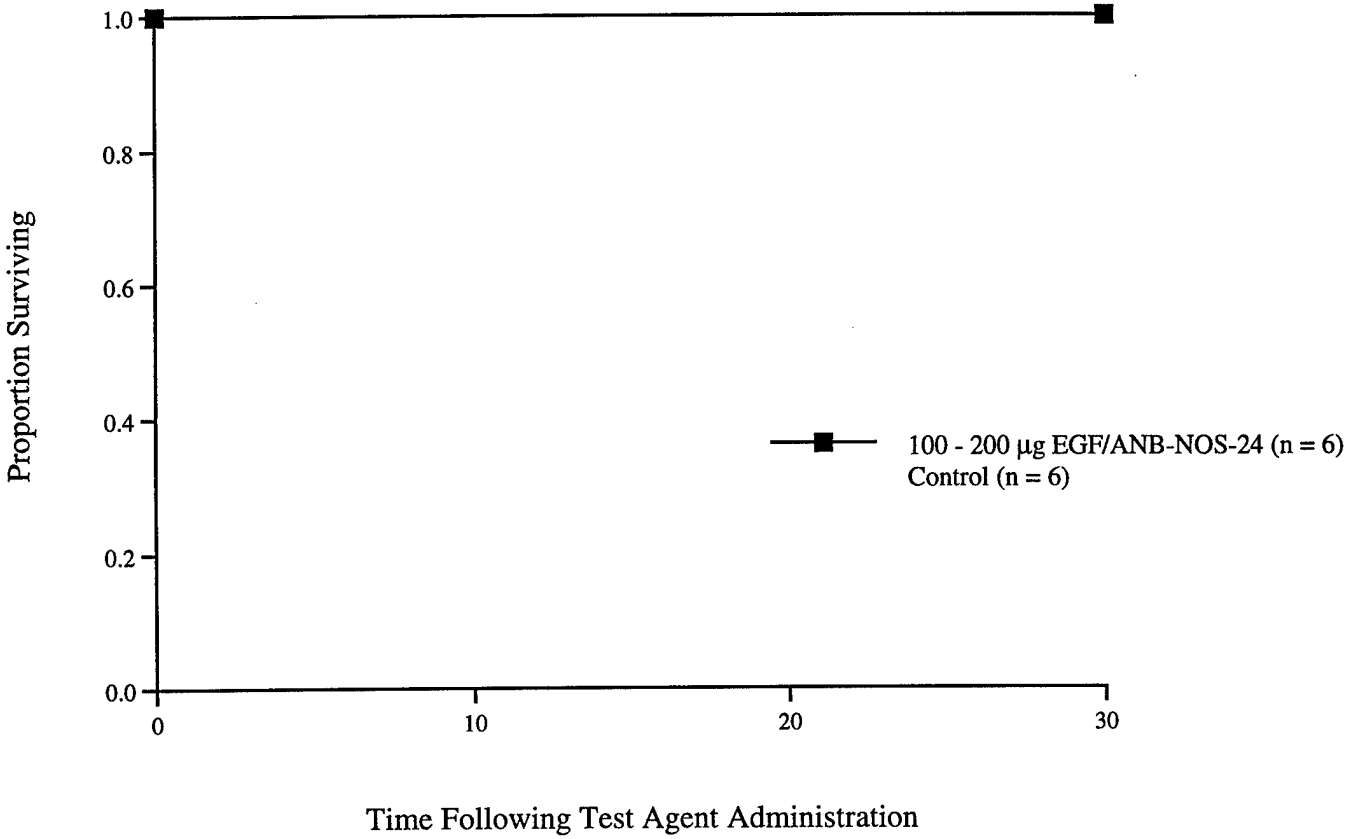


FIGURE 7i

Effect of EGF/ANB-NOS-41 on Survival of Balb/c Mice

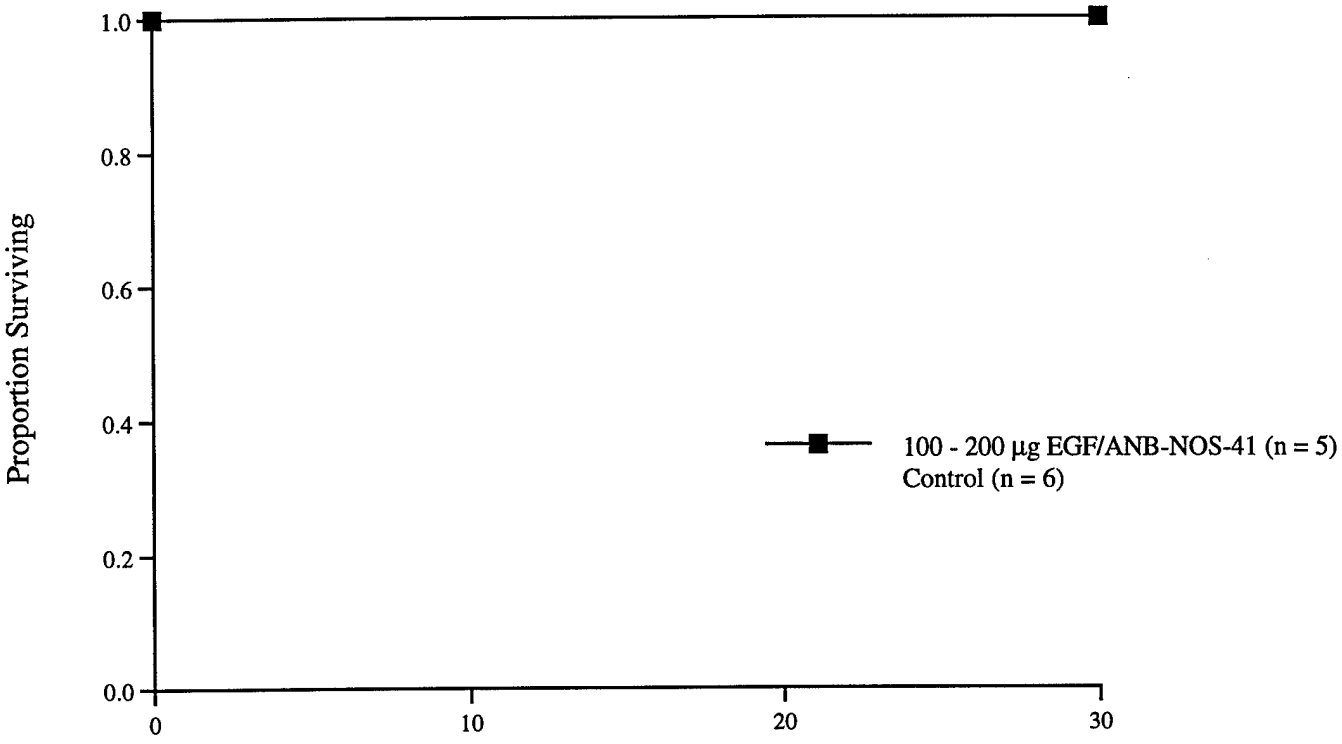


FIGURE 7j

Effect of EGF/ANB-NOS-Gen on Survival of Balb/c Mice