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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D.C. 20523

DATE: 8/4/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 5.140
1st PR

Attachment

PN-ABA-535

5.140

# FIRST PROGRESS REPORT OF RESEARCH PROJECT "EVALUATION IN VITRO OF INTERLEUKIN 1 AND INTERLEUKIN 2 AS POSSIBLE IMMUNOTHERAPEUTIC AGENTS IN LEPROSY"

SUBMITTED TO : U.S. Agency for International Development

GRANT NUMBER : 936-5542-G-00-5056-00

TOTAL PROJECT PERIOD : 06/10/1985-10/31/1987

PERIOD COVERED BY THIS REPORT : 06/10/1985-12/31/1985

PRINCIPAL INVESTIGATOR : Sanit Makonkawkeyoon, Ph.D.
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CO-INVESTIGATORS : Vicharn Vithayasai, M.D., Ph.D.
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Rec'd in SCI FEB 12 1986

1. Personnel Management and Activities :

Dr.Sanit Makonkawkeyoon, principal investigator, worked very closely with Dr.Vicharn Vithayasai, Co-investigator, to set up various methods for research studies in leprosy. These research techniques included separation of peripheral blood mononuclear leukocytes, nonspecific esterase staining of monocytes, separation and quantitation of monocytes, production and quantitation of IL1, separation and quantitation of T lymphocytes, production and quantitation of IL2. We have 4 hours of research meetings every week for discussion of various problems.

There are 4 part-time scientists in this research project, two M.S. and two B.S. graduates. One M.S. has responsibility for the production and determination of IL1, separation and quantitation of monocytes. The other M.S. scientist is responsible for the production and quantitation of IL2, separation and quantitation of T lymphocytes. One B.S. is assigned to assist in the cells mixing studies, including separation and purification of monocytes, T lymphocytes and B lymphocytes. Various purified cells are mixed in various proportions, and the amount of IL2 produced from these various cells mixing are determined. The other B.S. is assigned to assist in the work of production and isolation of mouse IL2 dependent spleen cells, and he also helps to work out some new techniques which are generated from our research work.

One full-time laboratory assistant is hired to look after glassware cleaning, sterilization of laboratory materials, and general assistance in the laboratory. One part-time typist is also hired to type records, research data, progress reports and manuscripts for publication.

2. Purchase of Equipment and Materials :

All listed equipment in this research project, such as liquid scintillation counter, centrifuge, cell harvester and vertical laminar flow hood have been ordered, and some have already arrived and are in use. The purchase of scientific materials, such as laboratory glassware, antisera, chemicals and supplies from a local agency is convenient, but rather expensive. Only some minor problems have been encountered.

3. Progress Report :

A. OBJECTIVES :

The overall objectives of this research project are :

(1) To determine the capability of monocytes and lymphocytes from leprosy patients and healthy subjects to produce IL1 and IL2 respectively.

(2) To assess the effect, in vitro, of exogenous IL1 and IL2 on lymphocytes from leprosy patients.

(3) Determination of cell types required for IL2 production.

B. ACTIVITES:

PART I. PRODUCTION AND DETERMINATION OF IL1

1. Separation and Purification of Human Monocytes.

Twenty milliliters of heparinized venous blood was obtained from subjects. The blood is diluted 1:2 with sterile 0.01 M phosphate buffer saline (PBS) pH 7.2 and centrifuged on Ficoll-Hypaque. The peripheral blood mononuclear leukocytes (PBMLS) is collected, washed 3 times with RPMI 1640 media, and the cell concentrations adjusted with RPMI 1640

containing 100 units/ml penicillin, 100 ug/ml streptomycin, 2 mM L-glutamine and 10% heat-inactivated fetal calf serum.

PBMLs $4 \times 10^6$ cells in 3 ml media were added onto a 60 mm. plastic plate, incubated at 37°C 5% $CO_2$ for 4 hrs. The non-adherent cells are washed off 3 times with 10% FCS in RPMI 1640. PBMLs, non-adherent and adherent cells are stained with nonspecific esterase stain (NSE).

2. Production and Quantitation of IL1.

2.1 Titration of Monocyte Concentrations.

The adhered monocytes on the plastic plate is adjusted to $3 \times 10^5$, $4 \times 10^5$, $5 \times 10^5$ cells/ml by adding various amount of 10% FCS-RPMI 1640 media. Lipopolysaccharide (LPS) is added 20 ug/ml, incubated at 37°C, 5% $CO_2$ for 24 hrs. Cell suspension is collected and centrifuged, supernatants assayed for IL1.

2.2 Titration of LPS Concentrations.

The adhered monocytes $3 \times 10^5$ cells/ml in petri dishes are added with LPS to the concentration of 20, 40 and 60 ug/ml. They are incubated at 37°C 5% $CO_2$ for 24 hrs., centrifuged, and the supernatants assayed for IL1.

2.3 Assay of IL1.

Thymocytes from 8 weeks old BALB/C inbred strain mice are used as the indicator cells in IL1 assay. Animals are killed by cervical dislocation and the thymus gland removed under sterile conditions. Thymocyte suspension is prepared by gently homogenizing the thymus gland in a sterile mesh with a sterile rubber plunger. The thymocytes are washed twice with IL1 media, counted, and then adjusted to be $3 \times 10^7$ cells/ml.

The samples for IL1 assay are diluted 1:2, 1:4, 1:8, 1:16 and 1:32 by IL1 media with a final volume of 600 ul for each dilution. One hundred microliters of thymocyte suspension is added to each tube, and thoroughly mixed. Thirty microliters of PHA-P (conc. 20 ug/ml) is added to each tube, and mixed.

All IL1 samples and dilutions are assayed in triplicate using 96 wells tissue culture plate. Two hundred microliters of the above mixture is pipetted into each well. The microtiter tissue culture plate is incubated at 37°C 5% $CO_2$ for 72 hours. Thymidine incorporation into DNA is measured by adding 25 ul. of tritiated thymidine (8 uCi/ml) into each well, mixed, and incubated 2 hours.

The culture are harvested onto glass microfiber filters using a semiautomatic cell harvester. The glass microfiber filters are dried at room temperature overnight and transferred into scintillation counting vials containing 7.0 ml. liquid scintillation fluid. All samples are counted in a liquid scintillation counter (LS 7000 Beckman Liquid Scintillation Counter, Beckman Scientific Instruments, Inc., Fullerton, CA 92634 USA).

## PART II. PRODUCTION AND DETERMINATION OF IL2.

### 1.) TITRATION OF OPTIMAL CONDITIONS FOR IL2 PRODUCTION.

#### 1.1 Titration of Optimum Con. A Concentration.

PBMLs 1 x $10^6$ cells/ml are stimulated with 5, 10, 20, 40, 60 and 120 ug of concanavalin A (Con A), incubated at 37°C 5% $CO_2$ for 12 hours. The cell cultures are harvested and centrifuged. Supernatants are added with 20 mg/ml of $\alpha$-methyl-D-mannoside, filtered sterile and stored at -20°C until used.

#### 1.2 Titration of Optimum PBMLs Concentration.

Various concentrations of PBMLs (0.25 x $10^6$, 0.5 x $10^6$, 1.0 x $10^6$ and 2.0 x $10^6$ cells/ml of IL1 media) are stimulated with 40 ug of Con A, and incubated 12 hours at 37°C 5% $CO_2$. Culture supernatants are harvested by centrifugation at 600 G, 15 min. The supernatants are added with 20 mg/ml of $\alpha$-methyl-D-mannoside, filtered sterile, and stored at 20°C.

#### 1.3 Titration of Optimum Incubation Time.

PBMLs (1 x $10^6$ cells/ml) are stimulated with 40 ug Con A, incubated for 4, 8, 12, 18, 24 and 48 hours. At the end of each culture period, culture supernatants are harvested, and then added with 20 mg/ml of $\alpha$-methyl-D-mannoside, filtered sterile, and stored at -20°C.

### 2.) ASSAY OF IL2 ACTIVITY.

#### 2.1 Production of Rat Factor (IL2)

Rat spleen cells (5 x $10^6$ cells/ml) are stimulated with 5 ug Con A, and incubated at 37°C 5% $CO_2$ for 48 hours. Culture

supernatant is harvested, added with 20 mM/ml of $\alpha$-methyl-D-mannoside, filtered sterile, and stored 2 ml aliquots in vial at -20°C.

2.2 Titration of Optimum Condition for IL2 Assay.

CTLL-2 cells, a continuous IL2 dependent line of cytotoxic T cells are used as indicator cells in the IL2 assay. This cell line is washed 3 times with IL1 media and resuspended at $2 \times 10^3$, $4 \times 10^3$, $6 \times 10^3$ and $8 \times 10^3$ cells/100 ul. Rat factor (IL2) is diluted in serial dilution from 1:2, 1:4, 1:8 to 1:256 in 100 ul. in each well of a 96 wells tissue culture plate. One hundred microliters of various concentrations of CTLL-2 cell suspension are added into each well of diluted rat factor, mixed, and incubated at 37°C 5% $CO_2$ for 24 hours. Fifty microliters containing 0.2 uCi of $^3$H-TdR are added into each well and incubated for another 4, 6 and 8 hours. Cultures are harvested onto glass fiber filters and $^3$H-TdR incorporation is determined in a liquid scintillation counter.

2.3 IL2 Assay.

Tested samples are diluted in serial dilution 1:2, 1:4 to 1:256 with IL1 media in 100 ul volume in each well of a 96 wells tissue culture plate. One hundred microliters of twice washed CTLL-2 containing $8 \times 10^3$ cells is added into each well, mixed, and incubated 24 hours. Fifty microliters of $^3$H-TdR containing 0.2 uCi are added into each well and incubated for 8 hours. Cultures are harvested onto glass fiber filters and $^3$H-TdR incorporation is determined by a liquid scintillation counter.

## 3.) MAINTAINACE AND PREPARATION OF CTLL-2.

### 3.1 Maintainance of CTLL-2.

CTLL-2 cells were obtained from Professor Dr.Ward E.Bullock, Division of Infectious Diseases, Department of Internal Medicine, College of Medicine, University of Cincinnati, U.S.A. The cell line at $5 \times 10^4$ cells/ml was grown in IL1 media supplement with 20% rat factor. At every 2 days, the cell line was split into $5 \times 10^4$ cells/ml and grown in 20% rat factor in IL1 media.

### 3.2 Preparation of CTLL-2 for IL2 Assay.

At the end of 2 days growth, the CTLL-2 cells are washed twice with IL1 media. The cell pellet is resuspended and incubated at 37°C 5% $CO_2$ for 1 hour. The supernatant is removed by centrifugation and the cell plellet is diluted to $1.0 \times 10^6$ cells/ml with IL1 medium. This cell suspension is now ready to be used in the IL2 assay.

## PART III. DETERMINATION OF CELL TYPES REQUIRED FOR IL2 PRODUCTION.

### 1) Role of Monocytes in IL2 Production.

Twenty milliliters of venous blood is obtained from normal subjects in a heparinized tube. PBMLs are separated by Ficoll-Hypaque density gradient centrifugation, washed and cell concentration adjusted to $1 \times 10^6$ cells/ml. PBMLs $3 \times 10^6$ cells/3 ml are added onto a 10 x 35 mm tissue culture dish, incubated 2 hours at 37°C 5% $CO_2$. Nonadherent cells are washed out 3 times with 5% FCS-RPMI. T cells in the nonadherent cells are AET-rosetted and separated by Ficoll-Hypaque gradient centrifugation. Various concentrations of purified T cells are added onto adherent cells which adhere on a plastic dish. 5% FCS-RPMI media is added with a final total volume of 2 ml. containing 10 or 20 ug Con A. IL2 from various cells co-culture is determined.

### 2) Role of T Cell Concentrations in IL2 Production.

Purified T cells is prepared by AET-rosetted of the nonadherent cells. Various concentrations of the purified T cells are stimulated with 10 and 20 ug. Con A for IL2 production. At one T cell concentration producing minimum amount of IL2 would be selected for mixing with various amounts of purified monocytes to produce maximum amount of IL2.

## PART IV. PRODUCTION AND ISOLATION OF MOUSE IL2-DEPENDENT SPLEEN CELLS.

### 1.) Production of Growth Medium (Rat IL2).

Rat spleen cells ($1 \times 10^6$ cells/ml) are stimulated with 5 ug/ml Con A, and incubated at 37°C 5% $CO_2$ for 48 hours. Culture supernatant is harvested, added with 20 mg/ml of $\alpha$-methyl-D-mannoside, filtered sterile, and stored 2 ml aliquots in vial at -20°C

### 2.) Induction and Isolation of Mouse IL2-dependent Lymphoid Cells.

Mouse (Balb/c) spleen cells are freshly prepared from the animals. The thrice washed spleen cells ($1 \times 10^6$ cells/ml) are stimulated with 5 ug/ml Con A, and incubated at 37°C 5% $CO_2$ for 72 hours. The Con A-stimulated spleen cells are harvested and washed twice with RPMI medium. The cells are adjusted to $5 \times 10^5$ cells/ml with IL1 medium supplemented with 20% growth medium, incubated at 37°C 5% $CO_2$ 48 hours. These cells are subcultured with 20% growth medium in IL1 medium every other day.

### 3.) Test of IL2-dependent Cells.

After mouse (Balb/c) spleen cells were stimulated with Con A for 3 days, The cells were washed with IL1 medium and adjusted to $5 \times 10^5$ cells/ml with IL1 medium only, IL1 medium supplemented with 5 ug/ml Con A, and IL1 medium supplemented with 20% growth medium. All cell cultures are incubated at 37°C 5% $CO_2$ for 48 hours. Total cell number is determined in a hemocytometer and the viable cell number determined by trypan blue exclusion.

C. RESULTS:

PART I. PRODUCTION AND DETERMINATION OF IL1.

Table 1. Shows results of separation and purification of normal human monocytes. The purity of nonspecific esterase positive cells (monocytes) is ranged from 74% - 82%.

Table 1. Separation and purification of human monocytes.

| Subject No. | %+ve NSE in PBML | %+ve NSE in non-adherent cells | % Cell Adhere | %+ve NSE in Adherent Cells |
|---|---|---|---|---|
| 1 | 12 | 6 | 20 | 82 |
| 2 | 13 | 2 | 14 | 78 |
| 3 | 10 | 1 | 8 | 82 |
| 4 | 14 | 2 | 16 | 74 |

Table 2 and figures 1,2,3 show results of monocytes concentrations for the production of maximum IL1. It is found that monocyte concentration at $3 \times 10^5$/ml producing maximum amount of IL1.

Table 2. Determination of monocyte concentrations for IL1 production.

| Monocyte conc. (cells/ml) | Sample No. | IL-1 activity (cpm) | | | | | | Thy + PHA | Thy only |
|---|---|---|---|---|---|---|---|---|---|
| | | IL1 Dilutions | | | | | nonstimulated | | |
| | | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:2 | | |
| $3 \times 10^5$ | 1 | 13,812 | 15,635 | 9,609 | 2,579 | 2,087 | 9,447 | 281 | 2,068 |
| | 2 | 12,111 | 11,455 | 7,645 | 4,905 | 2,377 | 11,604 | 281 | 2,068 |
| | 3 | 5,006 | 9,038 | 3,616 | - | 2,716 | 4,324 | 1,401 | 1,612 |
| $4 \times 10^5$ | 1 | 12,482 | 10,404 | 9,997 | 4,159 | 2,891 | 11,983 | 281 | 2,068 |
| | 2 | 12,099 | 11,974 | 6,753 | 5,374 | 1,601 | 9,784 | 281 | 2,068 |
| | 3 | 4,881 | 7,019 | 3,638 | 2,254 | 689 | 3,313 | 1,401 | 1,612 |
| $5 \times 10^5$ | 1 | 9,650 | 12,955 | 9,194 | 8,527 | 2,415 | 7,964 | 281 | 2,068 |
| | 2 | 4,596 | 10,154 | 8,473 | 8,007 | 4,740 | 5,293 | 281 | 2,068 |
| | 3 | 5,243 | 5,705 | 3,866 | 705 | - | - | 1,401 | 1,612 |
| Std. | 1 | 34,496 | 31,097 | 22,006 | 12,009 | 4,795 | 27,358 | 281 | 2,068 |
| | 2 | 34,496 | 31,097 | 22,006 | 12,009 | 4,795 | 27,358 | 281 | 2,068 |
| | 3 | 18,701 | 5,519 | 9,329 | 5,157 | 1,532 | - | 1,401 | 1,612 |

Table 3. and figures 4,5,6 depict the LPS concentrations for maximum stimulation of IL1 production. The appropriate LPS concentration for maximum stimulation of IL1 production is 20 ug/ml.

Table 3. Determination of LPS concentration for maximum stimulation of IL1 production.

| LPS conc. (ug/ml) | Sample No. | IL-1 activity (cpm) | | | | | | Thy+ PHA | Thy only |
|---|---|---|---|---|---|---|---|---|---|
| | | IL1 Dilutions | | | | | nonstimulated | | |
| | | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:2 | | |
| 20 | 1 | 13,831 | 8,288 | 5,479 | 5,199 | 2,337 | 13,339 | 1,026 | 726 |
| | 2 | 8,265 | 3,646 | 2,628 | 1,792 | 1,992 | 4,717 | 1,026 | 726 |
| | 3 | 4,205 | 3,469 | 5,461 | 5,041 | 2,111 | 3,701 | 1,211 | 1,159 |
| 40 | 1 | 1,350 | 2,104 | 2,171 | 1,899 | 892 | 1,115 | 1,026 | 726 |
| | 2 | 12,014 | 9,543 | 3,353 | 3,117 | 1,381 | 3,551 | 1,026 | 726 |
| | 3 | 3,018 | 3,859 | 2,981 | 2,063 | 1,969 | 4,865 | 1,211 | 1,159 |
| 60 | 1 | 3,900 | 3,491 | 2,198 | 1,329 | 1,560 | 2,478 | 1,026 | 726 |
| | 2 | 6,838 | 4,980 | 2,904 | 2,872 | 2,371 | 1,864 | 1,026 | 726 |
| | 3 | 1,645 | 2,679 | 2,316 | 1,223 | 2,144 | 3,624 | 1,211 | 1,159 |
| Std. | 1 | 39,221 | 24,673 | 6,173 | 1,816 | 462 | 30,700 | 1,026 | 726 |
| | 2 | 37,151 | 24,196 | 9,918 | 5,970 | 4,115 | 34,637 | 1,211 | 1,159 |

## PART II. PRODUCTION AND DETERMINATION OF IL2.

### 1. TITRATION OF OPTIMAL CONDITIONS FOR IL2 PRODUCTION.

#### 1.1 Titration of Optimum Con A Concentration.

Titration curves for Con A concentration to stimulate maximum production of IL2 are shown in figures7,8,9 and 10. The optimum concentration of Con A is found to be 40 ug/ml.

1.2 Determination of Optimum PBMLs Concentration.

The optimum PBMLs concentration for the production of maximum IL2 is depicted in figures 11,12,13 and 14. The optimum PBMLs concentration for maximum IL2 production can be 1.0 x $10^6$ cells/ml or 2 x $10^6$ cells/ml. However, the 1 x $10^6$ cells/ml was selected because blood specimens from subjects have to be limited to the minimum.

1.3 Titration of Optimum Incubation Time.

Figures 15,16,17,18,19,20.21,22,23,24,25,26 and 27 show the titration curves of optimum incubation time. Although after 8,12 and 18 hrs of incubation, all produced large amount of IL2, the incubation time of 8 hrs is chosen because at this point there is less variation of the amount of IL2 production than at other points.

2. ASSAY OF IL2 ACTIVITY.

2.1 Titration of Optimum Concentration of CTLL-2.

Titration curves for optimum concentration of CTLL-2 are depicted in figures 28,29 and 30. The appropriated concentration of CTLL-2 for IL2 assay is 8 x $10^3$ cells/well.

2.2 Effect of Con A to CTLL-2.

Figure 31 show various concentrations of Con A to CTLL-2 cell line. It can be concluded that Con A has no effect on CTLL-2.

PART III. DETERMINATION OF CELL TYPES REQUIRED FOR IL2 PRODUCTION.

Table 4 show effect of monocytes and T lymphocytes on the IL2 production. Monocytes alone produce very small amount of IL2, however, when homologous T cells was added, large amount of IL2 produced. T cells alone can produce lower amount of IL2.

Table 4. Effect of monocytes and T lymphocytes on the production of IL2.

| Cell Types | Exmperiment Number | | | | | |
|---|---|---|---|---|---|---|
| | 1 (cpm) | 2 (cpm) | 3 (cpm) | 4 (cpm) | 5 (cpm) | 6 (cpm) |
| mø only | 2,608 | 888 | 2,827 | 254 | 3,145 | 932 |
| mø + 4.5 x $10^6$ T | 13,419 | 9,727 | ND | ND | 15,172 | ND |
| 4.5 x $10^6$ T | 13,563 | ND. | ND | ND | 12,422 | ND |
| mø + 3.0 x $10^6$ T | 11,340 | 10,120 | ND | ND | 14,234 | 14,256 |
| 3.0 x $10^6$ T | ND. | ND. | ND | ND | 12,684 | 14,033 |
| mø + 1.5 x $10^6$ T | 5,805 | 6,651 | 6,272 | 3,477 | 15,498 | 14,064 |
| 1.5 x $10^6$ T | ND. | 5,808 | 3,870 | 2,157 | 7,915 | 8,047 |
| 3 x $10^6$ PBML | 16,622 | 17,159 | 21,085 | 22,353 | 14,542 | 13,890 |

Table 5 showed the determination of concentration of T lymphocytes alone to produce minimum amount of IL2.

Table 5. Concentration of T lymphocytes produce minimum amount of IL2.

| Amount of T cells | Conc. of Con A | Amount of IL2 in Experiment 1 (cpm) | Amount of IL2 in Experiment 2 (cpm) |
|---|---|---|---|
| $1 \times 10^6$ | 20 | 237 | 2,015 |
| $1 \times 10^6$ | 10 | 2,276 | 230 |
| $5 \times 10^6$ | 20 | 520 | 1,006 |
| $5 \times 10^6$ | 10 | 1,018 | 847 |
| $2.5 \times 10^6$ | 20 | 1,198 | 2,164 |
| $2.5 \times 10^6$ | 10 | 112 | 557 |
| $2 \times 10^6$ PBMLs | 20 | 12,074 | 16,500 |

## PART IV. PRODUCTION AND ISOLATION OF MOUSE IL2-DEPENDENT SPLEEN CELLS.

Figures 32, 33 and 34 show the results of production and isolation of mouse IL2-dependent spleen cells. From the cell growth curves and percent of viable cells, it can be concluded that the mouse IL2-dependent spleen cells can be produced and isolated in our laboratory.

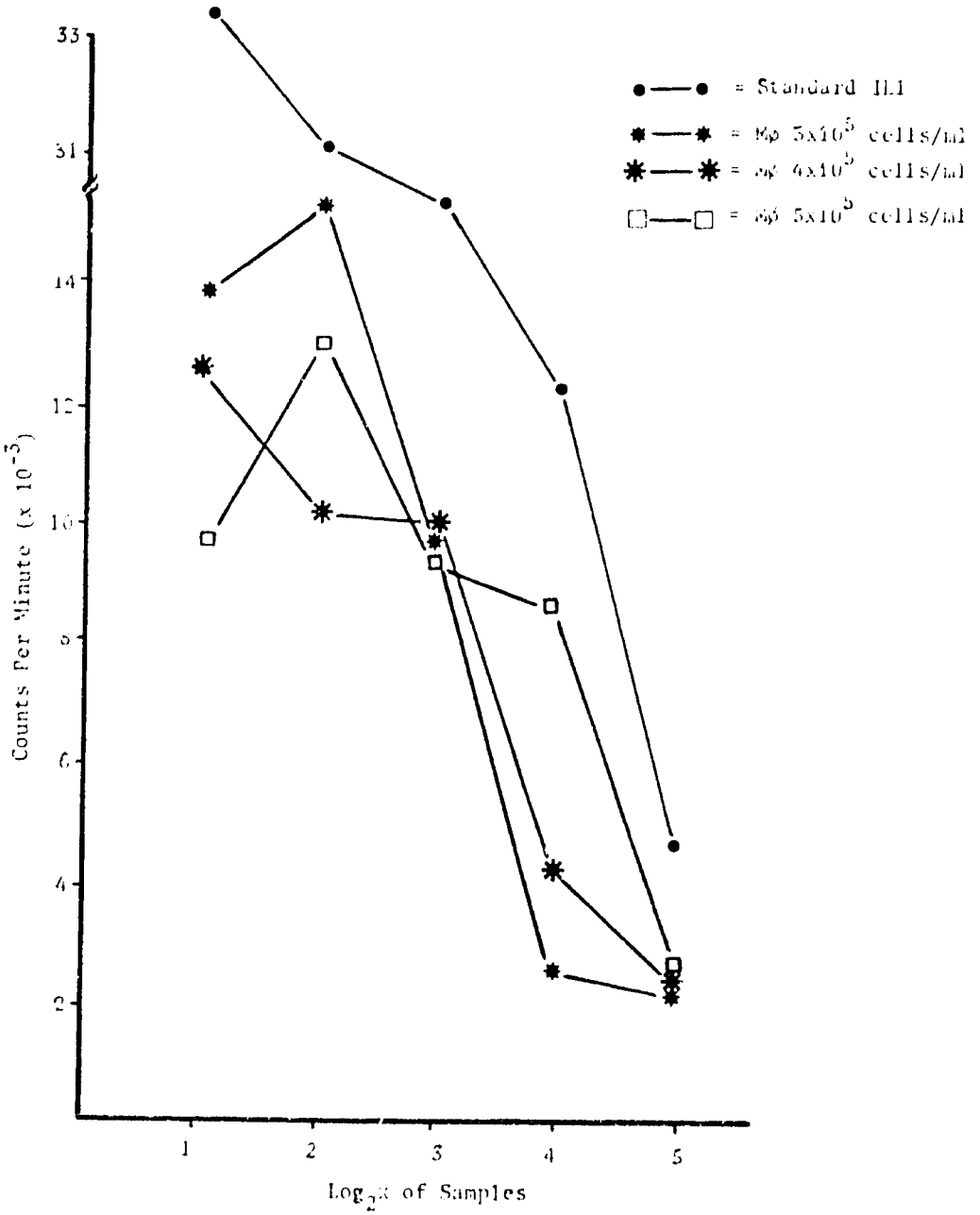


Figure 1. Monocyte concentrations for the production of IL1

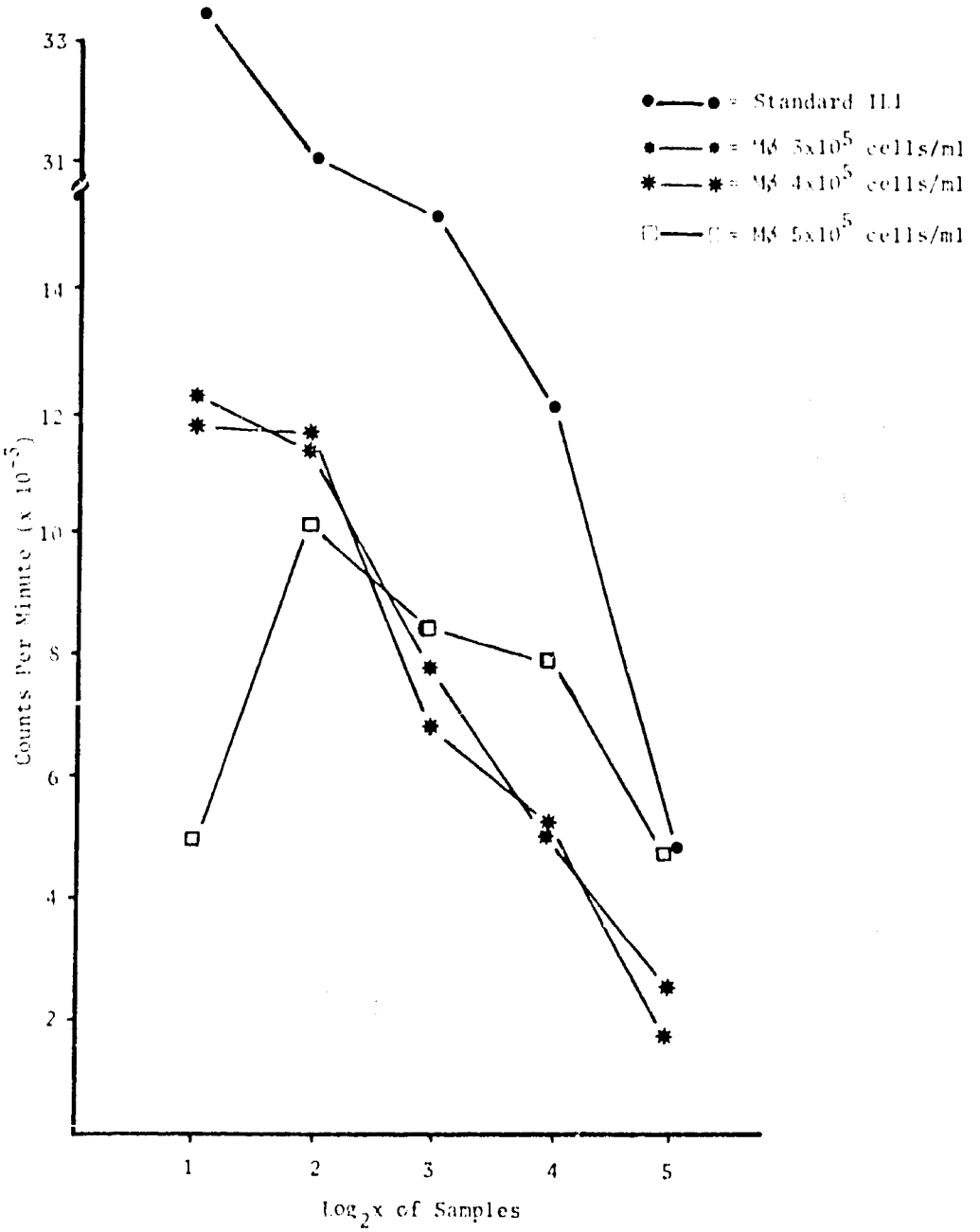


Figure 2. Monocyte concentrations for the production of IL1 (cont.)

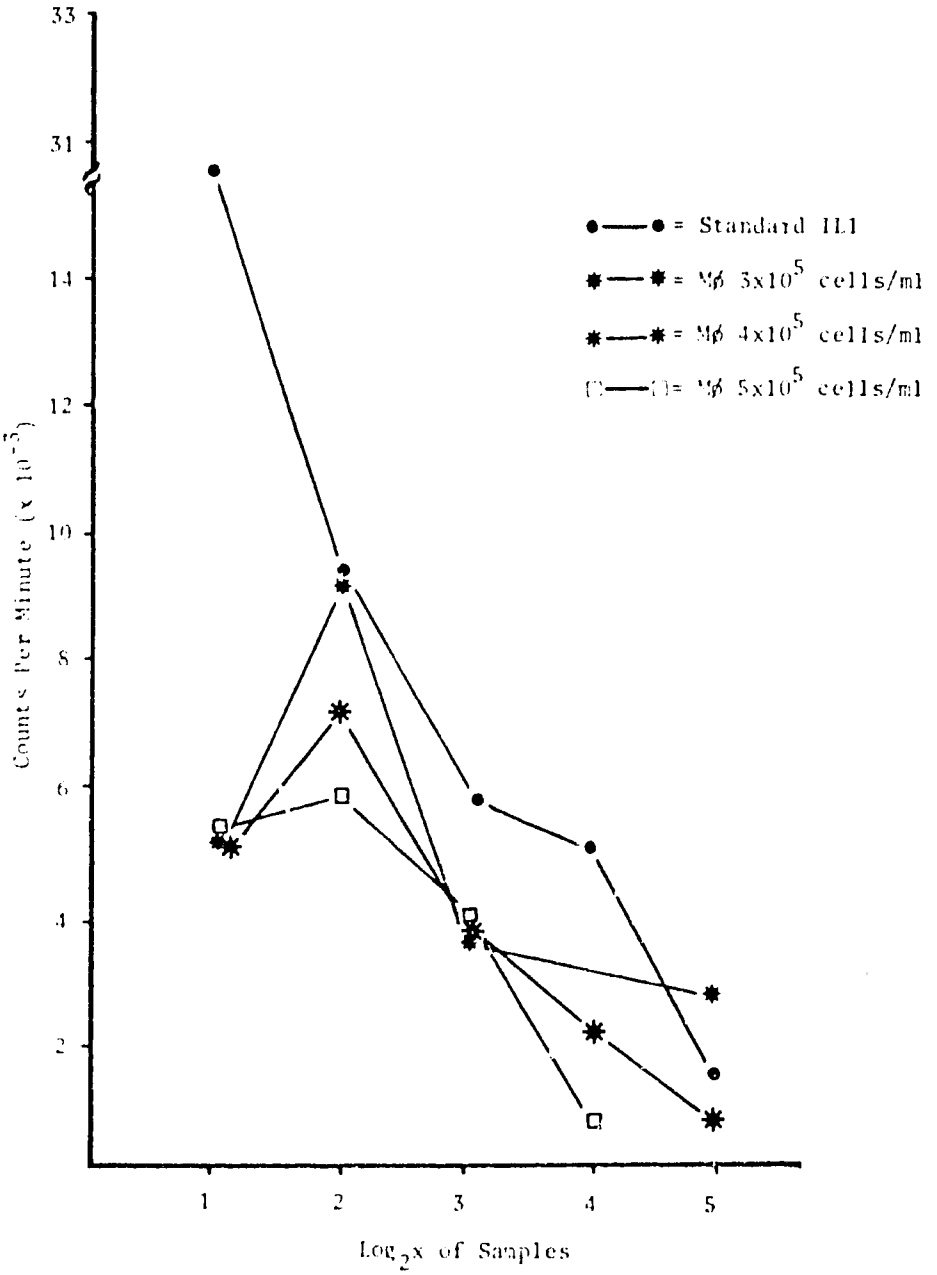


Figure 3. Monocyte concentrations for the production of IL1 (cont.)

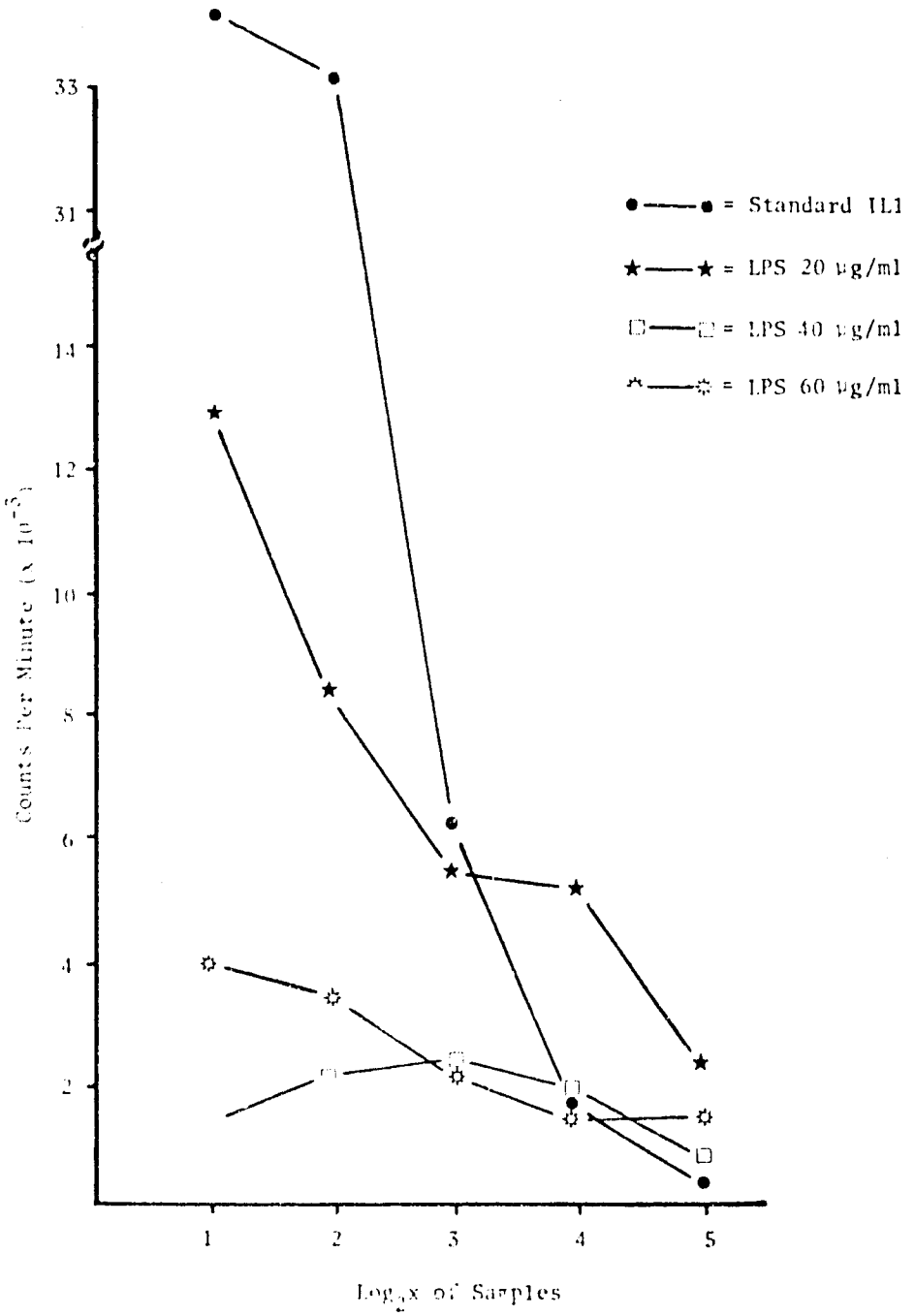


Figure 4. LPS concentrations for optimum stimulation of IL1 production.

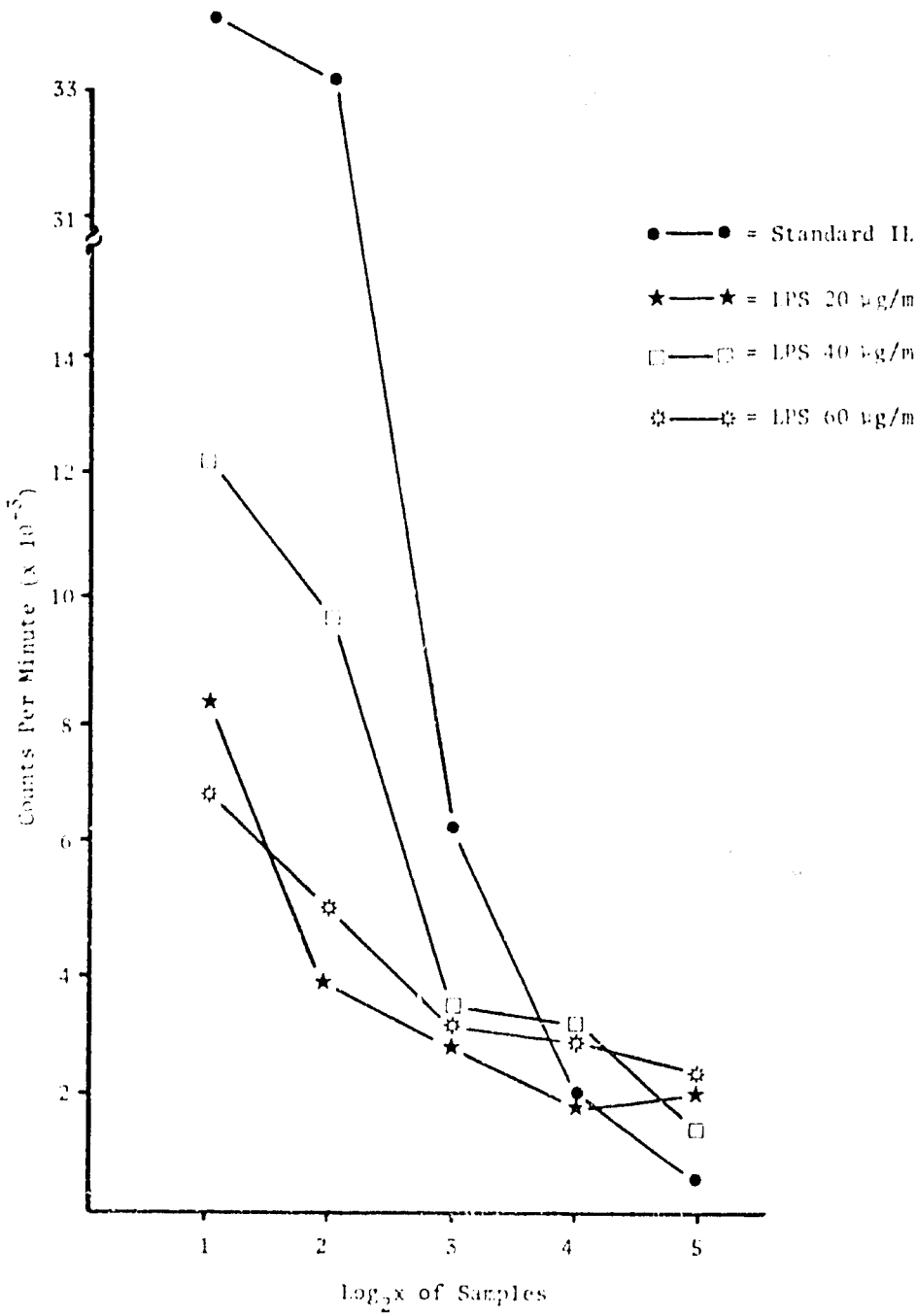


Figure 5. LPS concentrations for optimum stimulation of IL1 production (cont.)

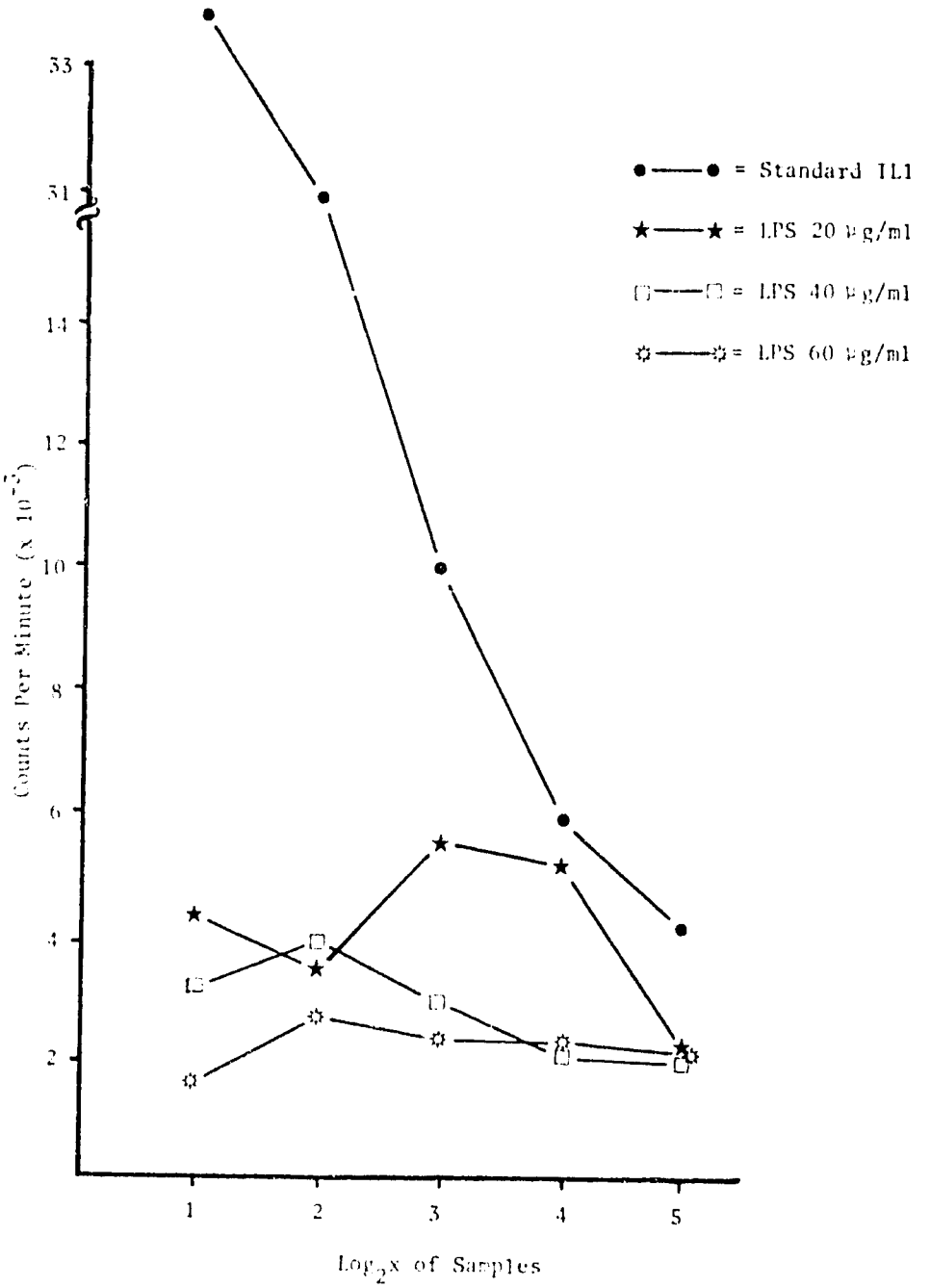


Figure 6. LPS concentrations for optimum stimulation of IL1 production (cont.)

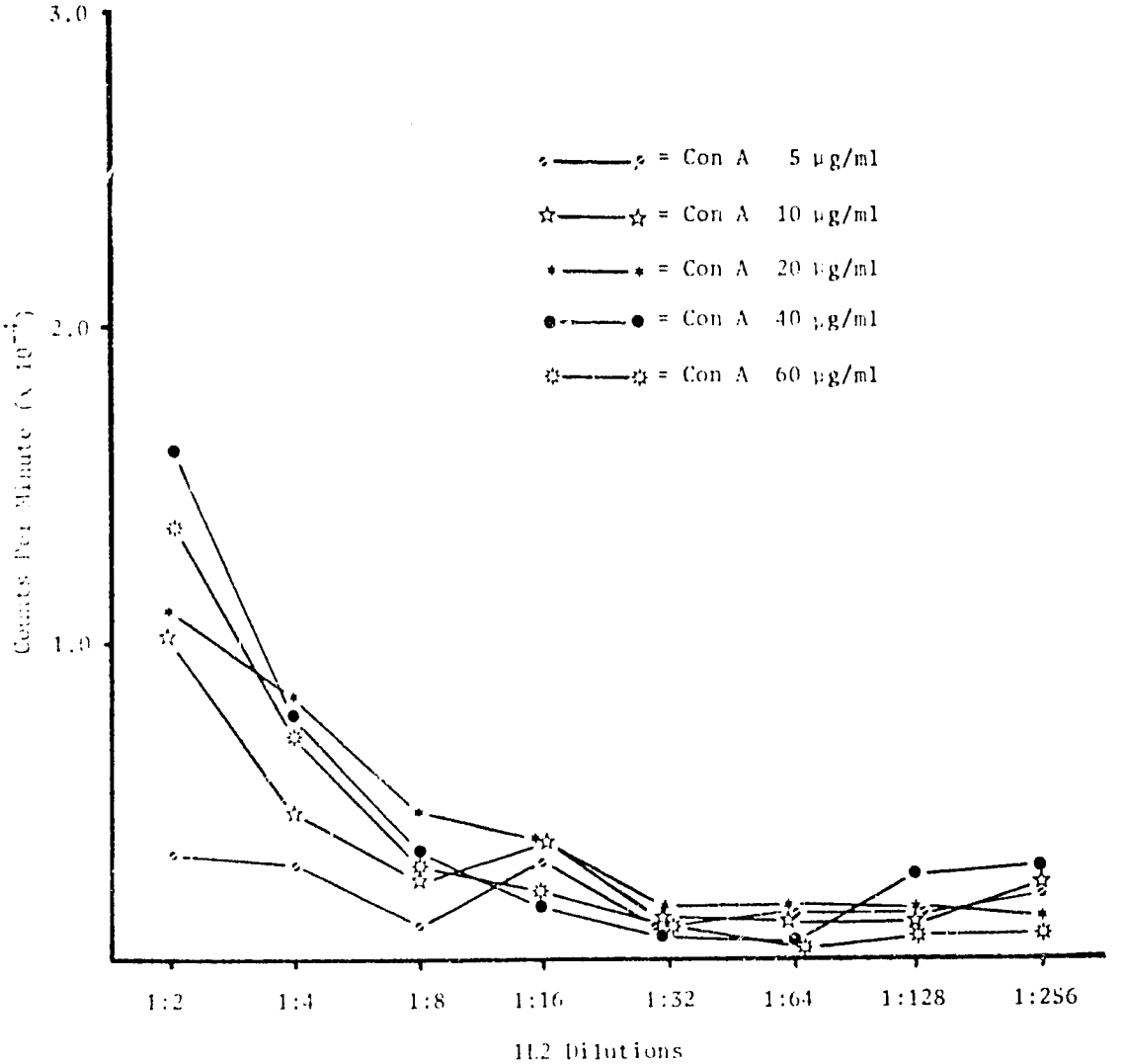


Figure 7. Concentration of Con A for optimum stimulation of IL2 production.

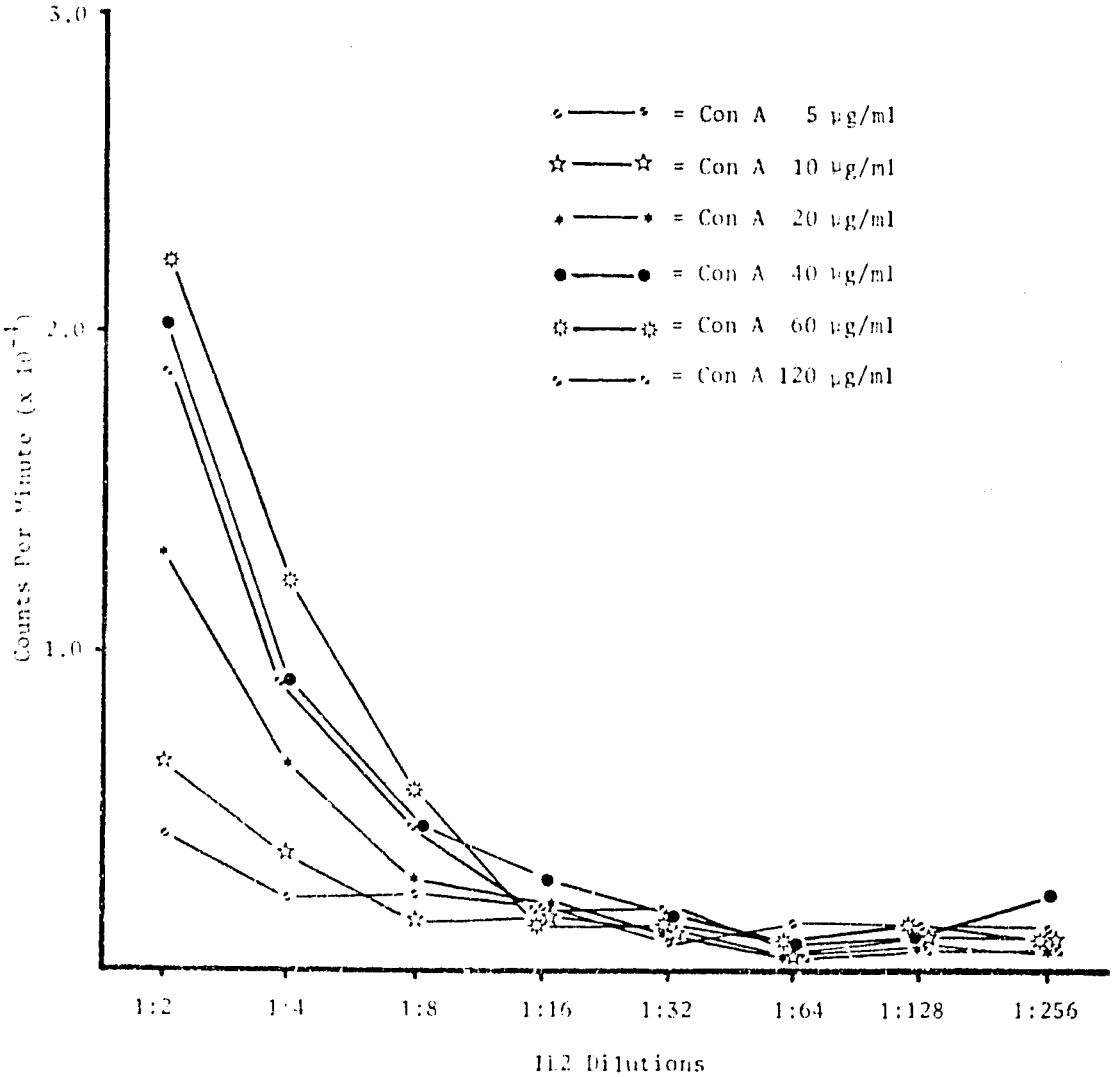


Figure 8. Concentrations of Con A for optimum stimulation of IL2 production (cont.)

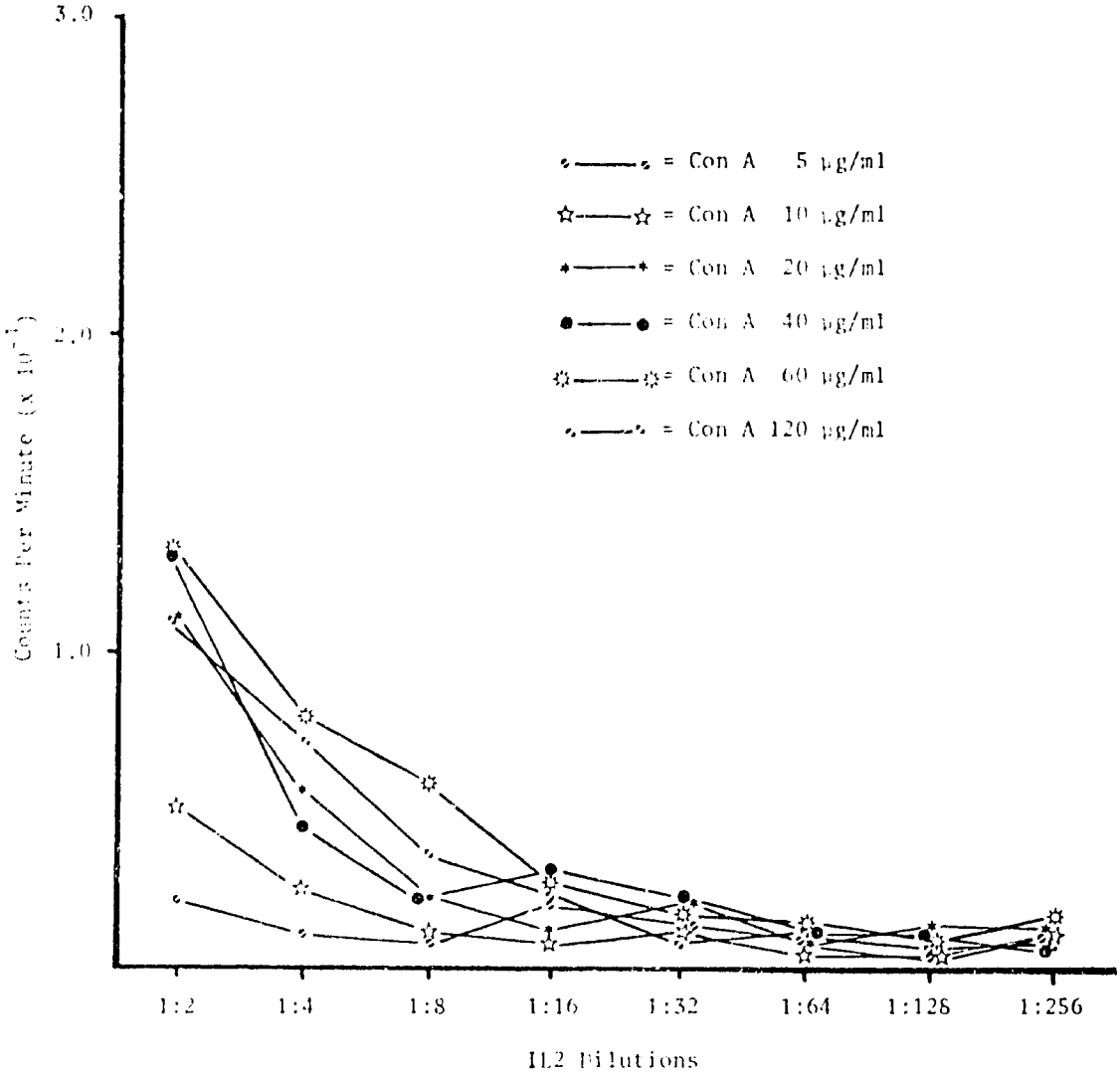


Figure 9. Concentrations of Con A for optimum stimulation of IL2 production (cont.)

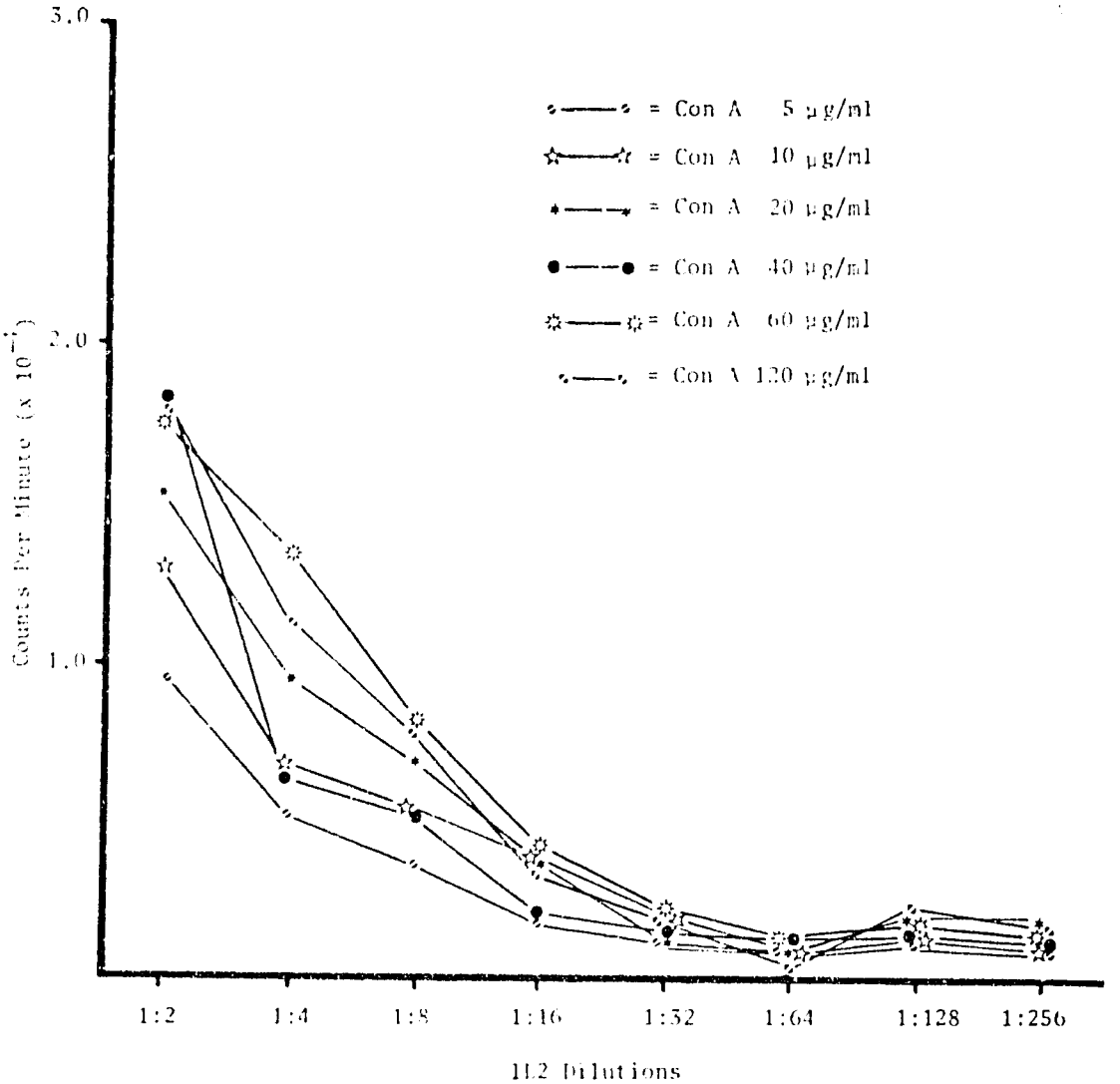


Figure 10. Concentrations of Con A for optimum stimulation of IL2 production (cont.)

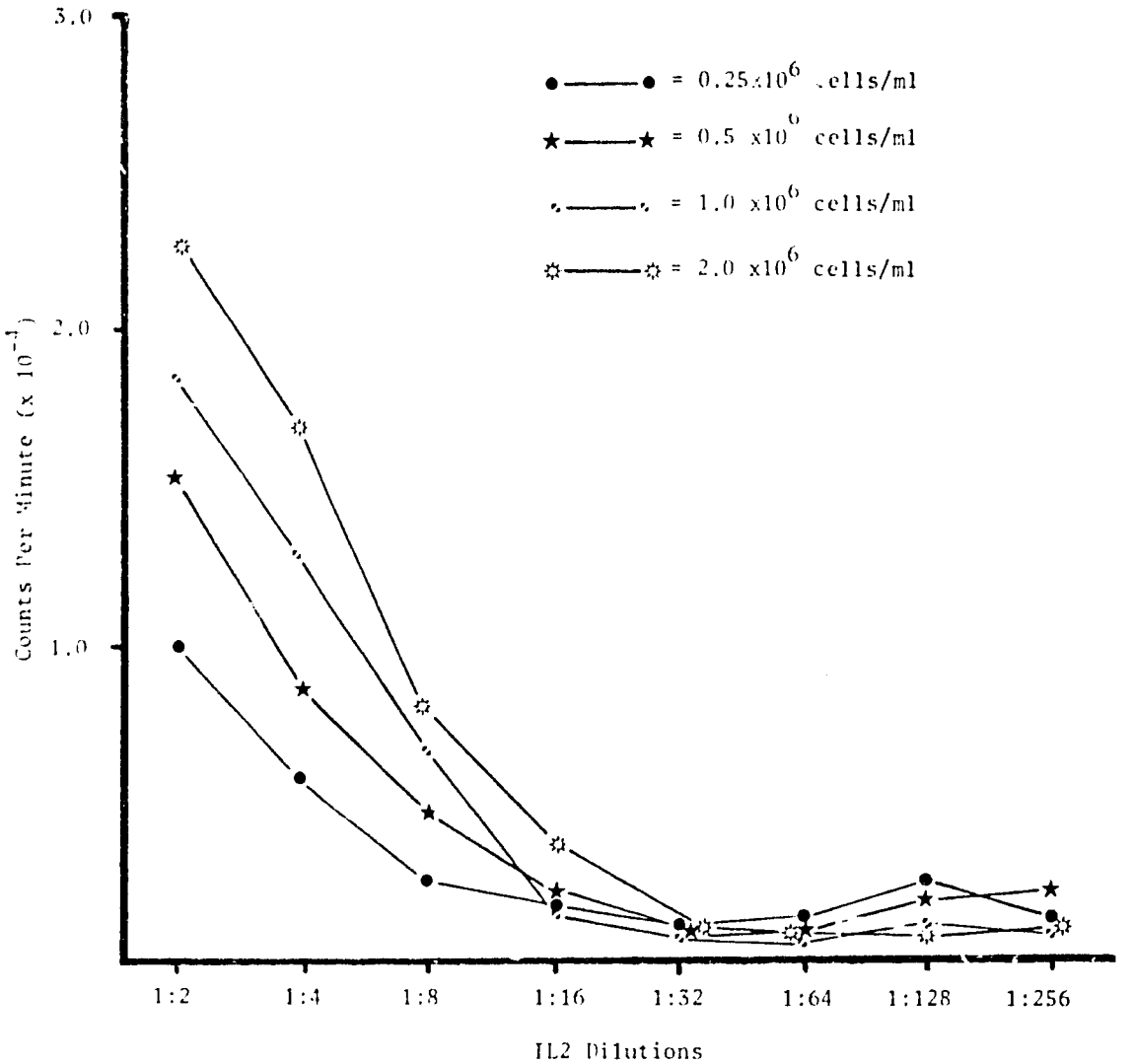


Figure 11. PBMLs concentrations for optimum production of IL2

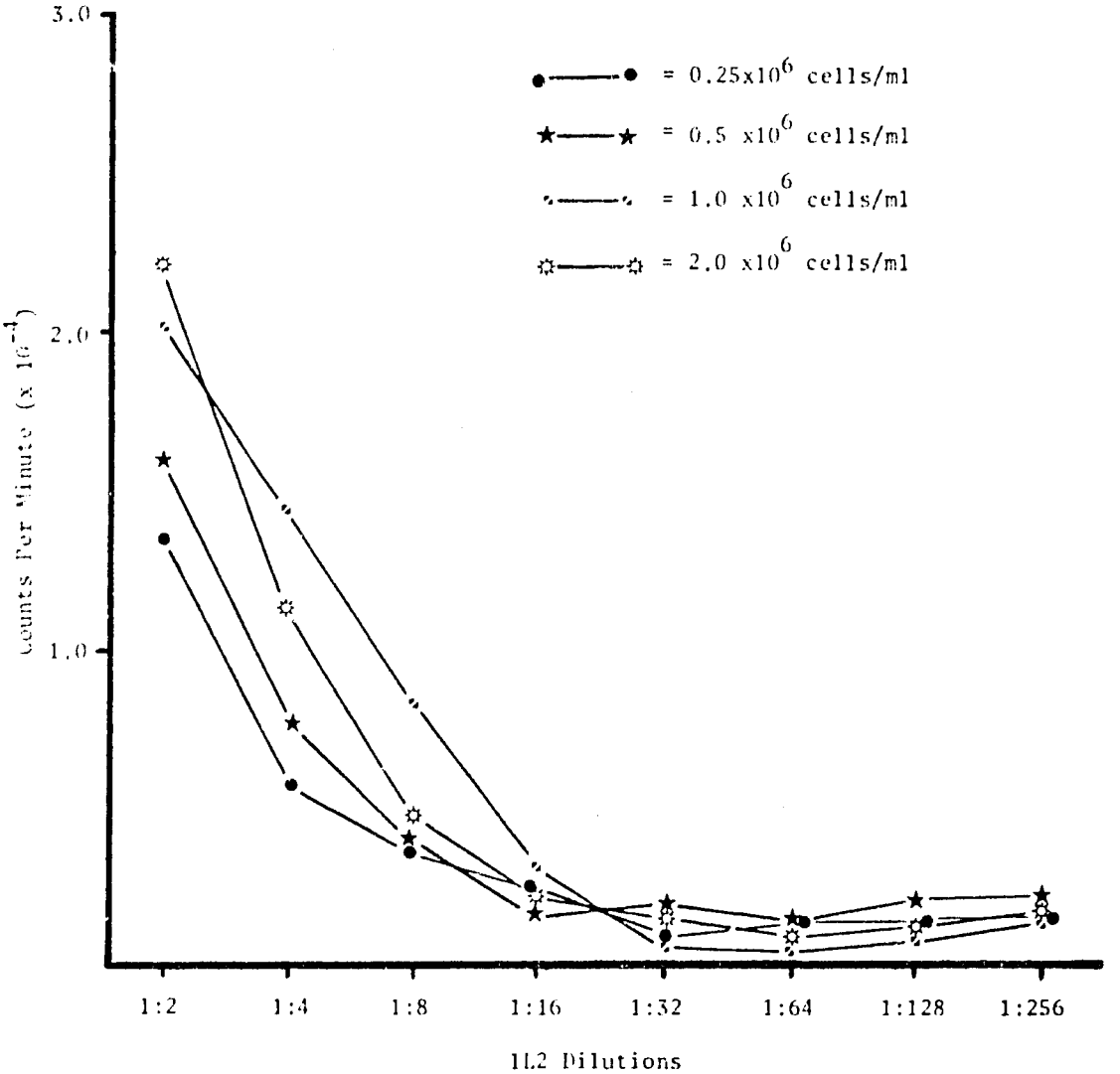


Figure 12. PBMLs concentrations for optimum production of IL2 (cont.)

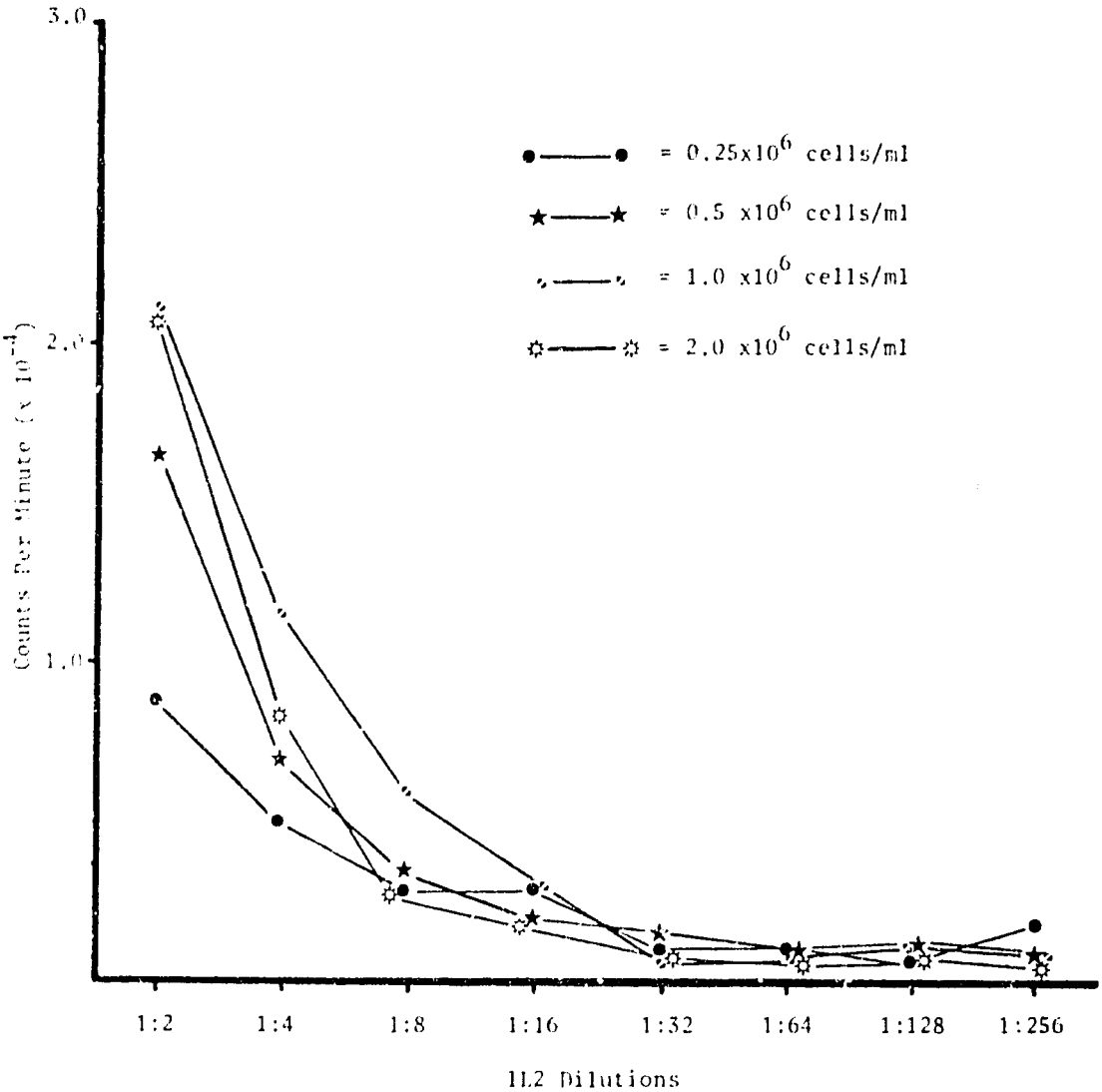


Figure 13. PBMLs concentrations for optimum production of IL2 (cont.)

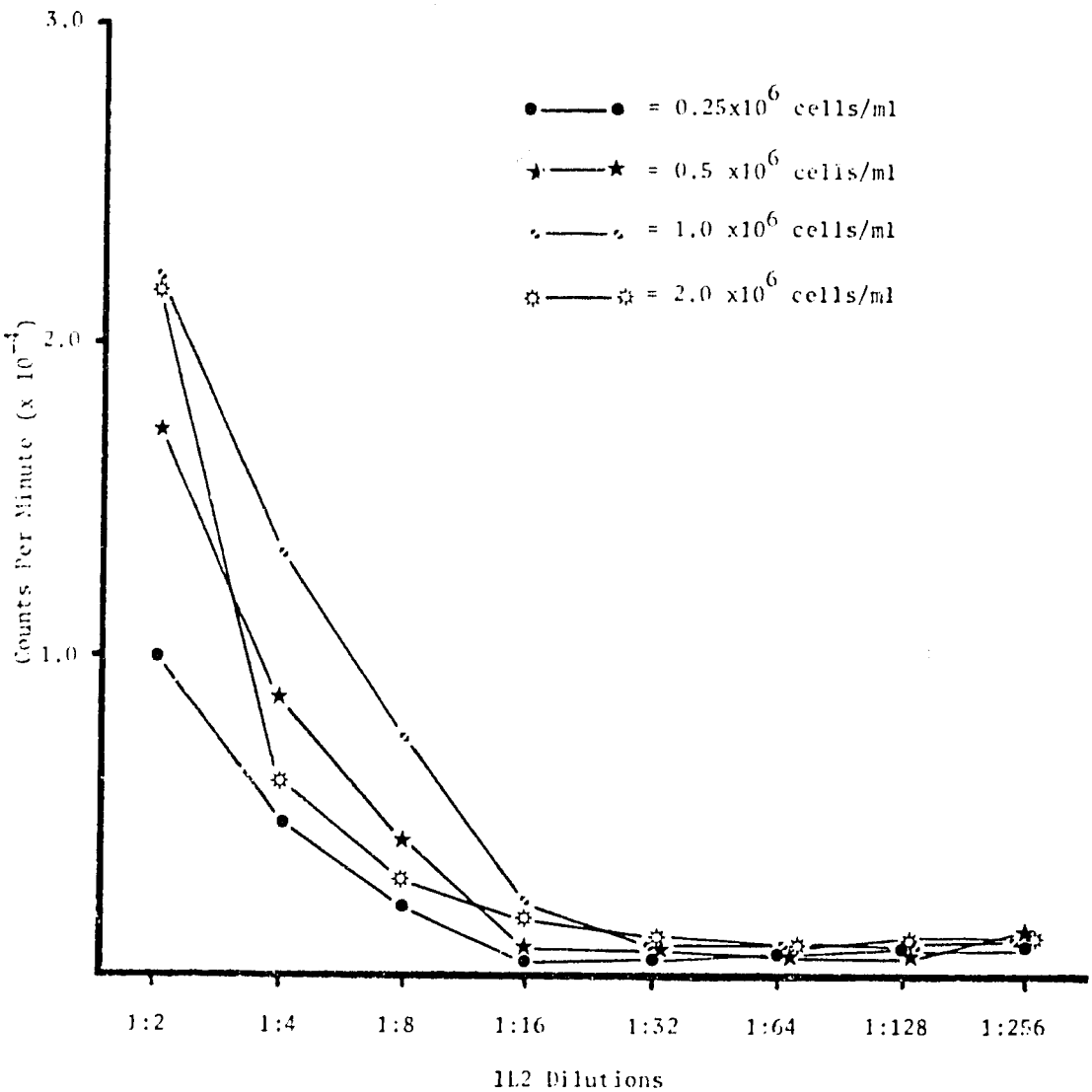


Figure 14. PBMLs concentrations for optimum production of IL2 (cont.)

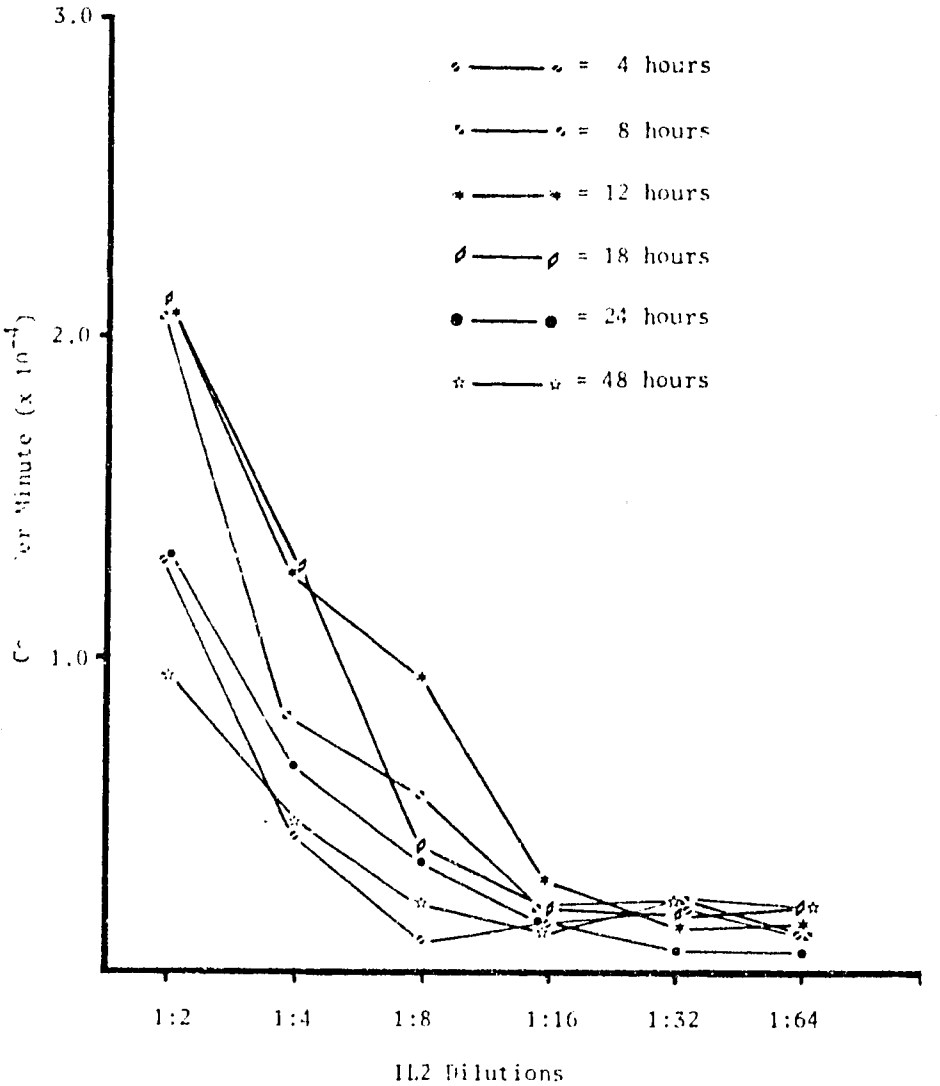


Figure 15. Optimum incubation time for IL2 production.

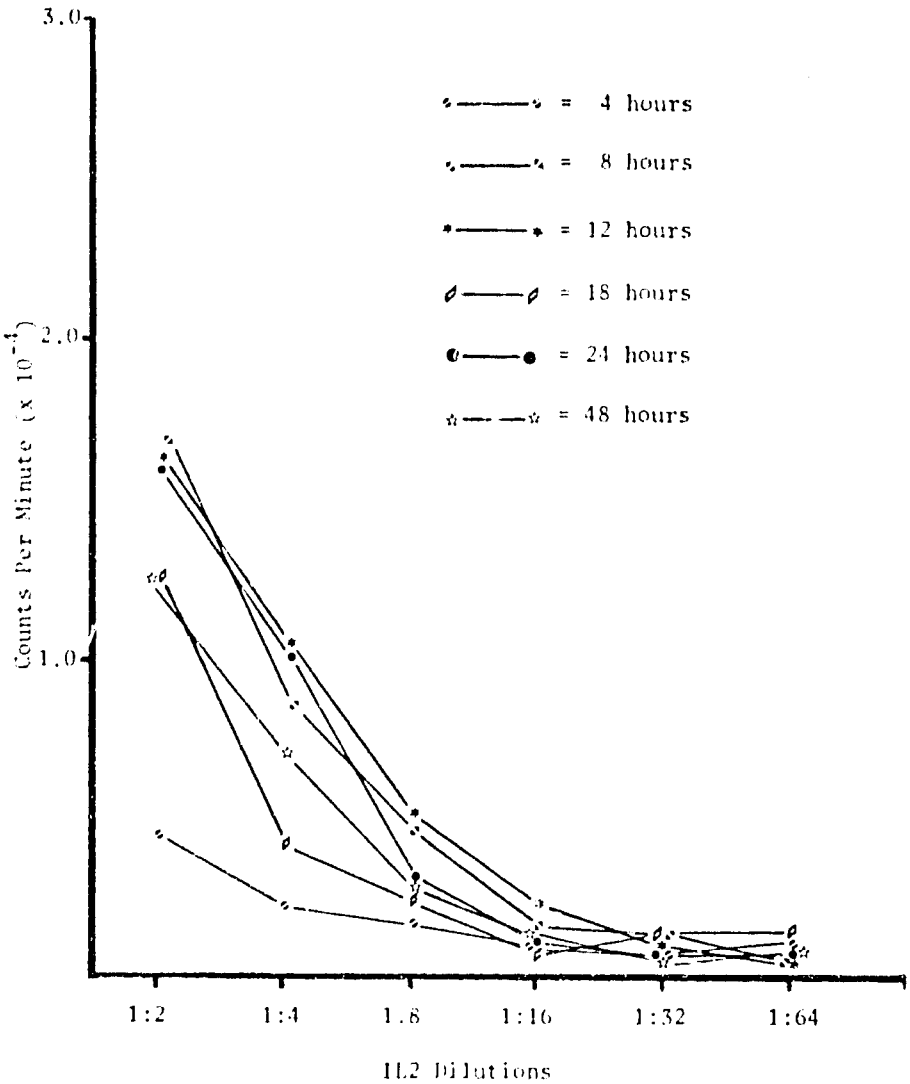


Figure 16. Optimum incubation time for IL2 production (cont.)

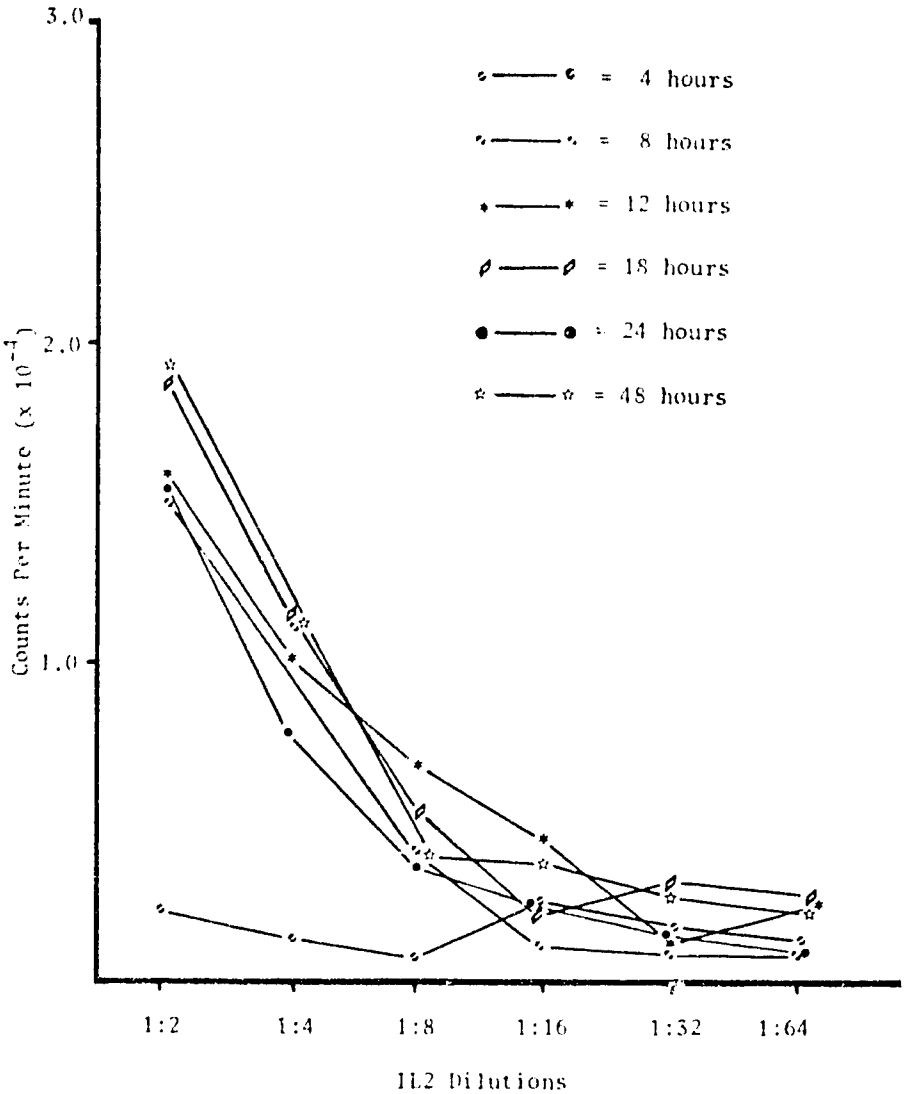


Figure 17. Optimum incubation time for IL2 production (cont.)

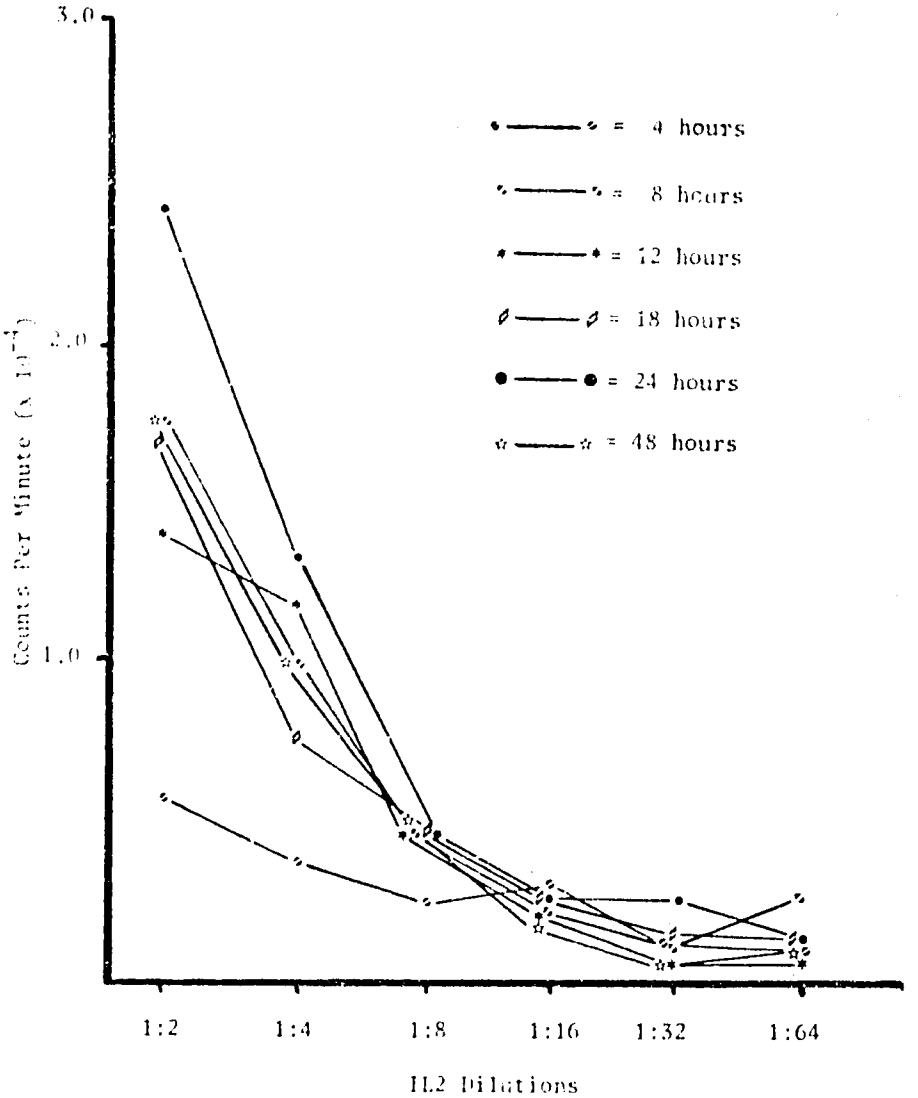


Figure 18. Optimum incubation time for IL2 production (cont.)

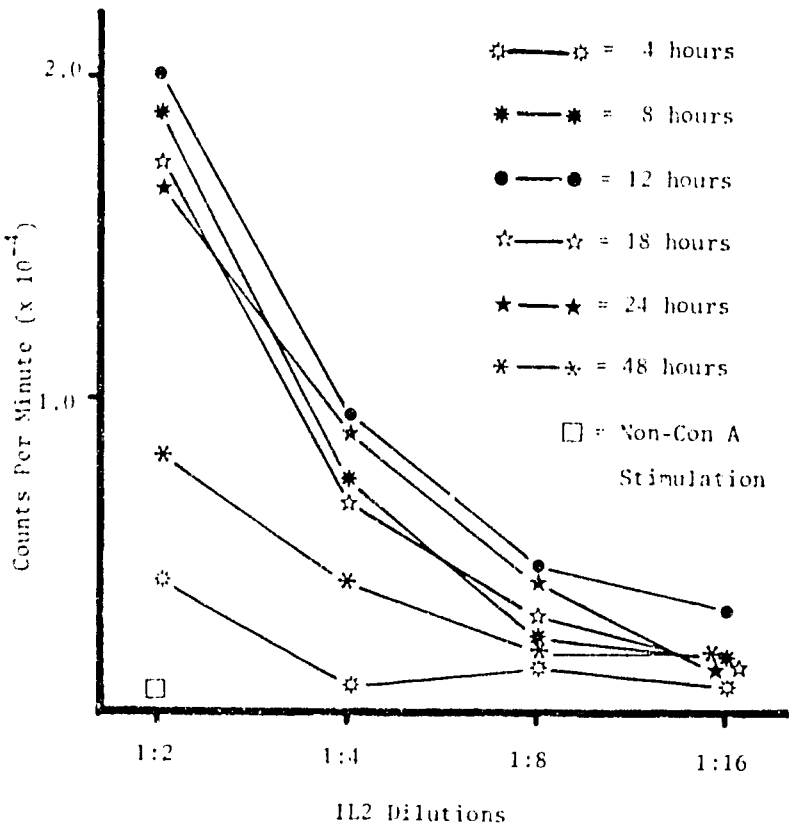


Figure 19. Optimum incubation time for IL2 production (cont.)

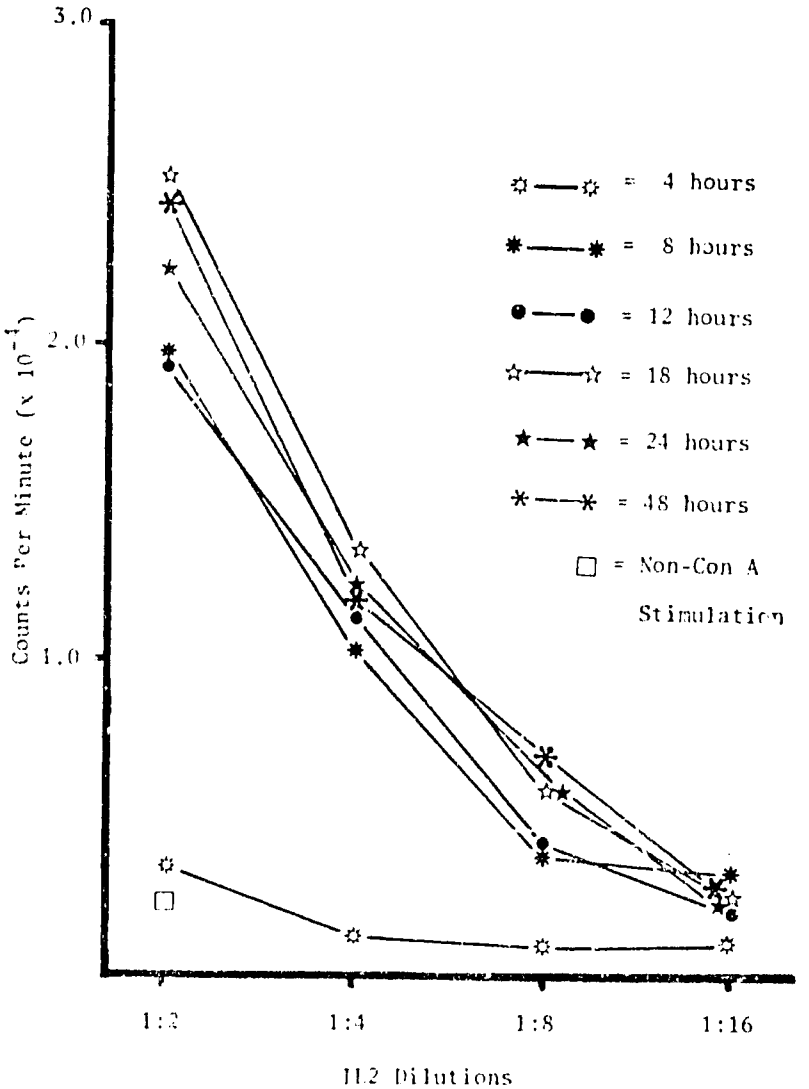


Figure 20. Optimum incubation time for IL2 production (cont.)

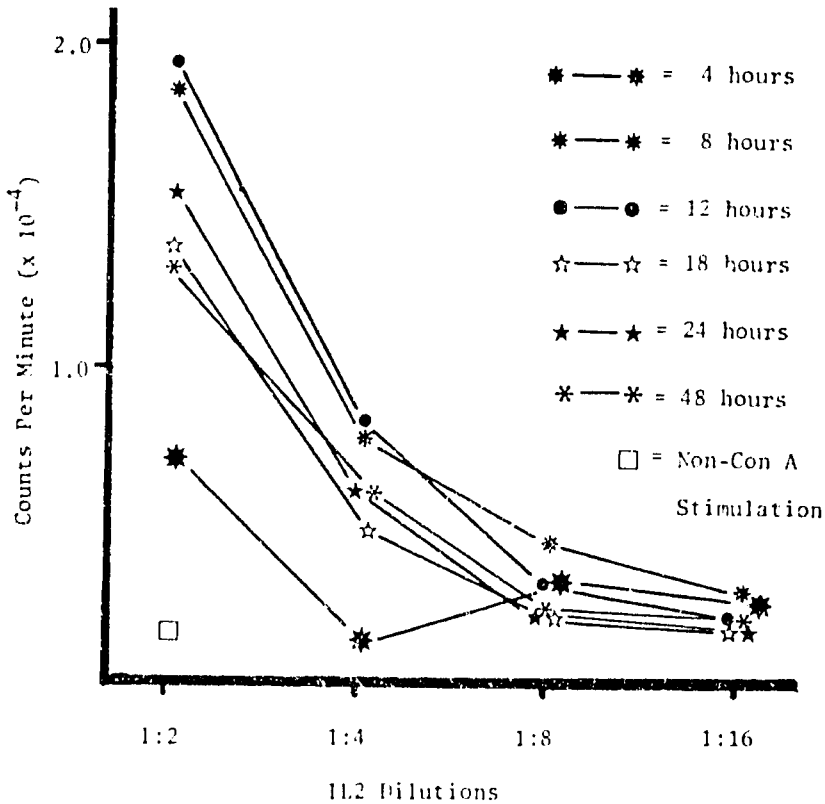


Figure 21. Optimum incubation time for IL2 production (cont.)

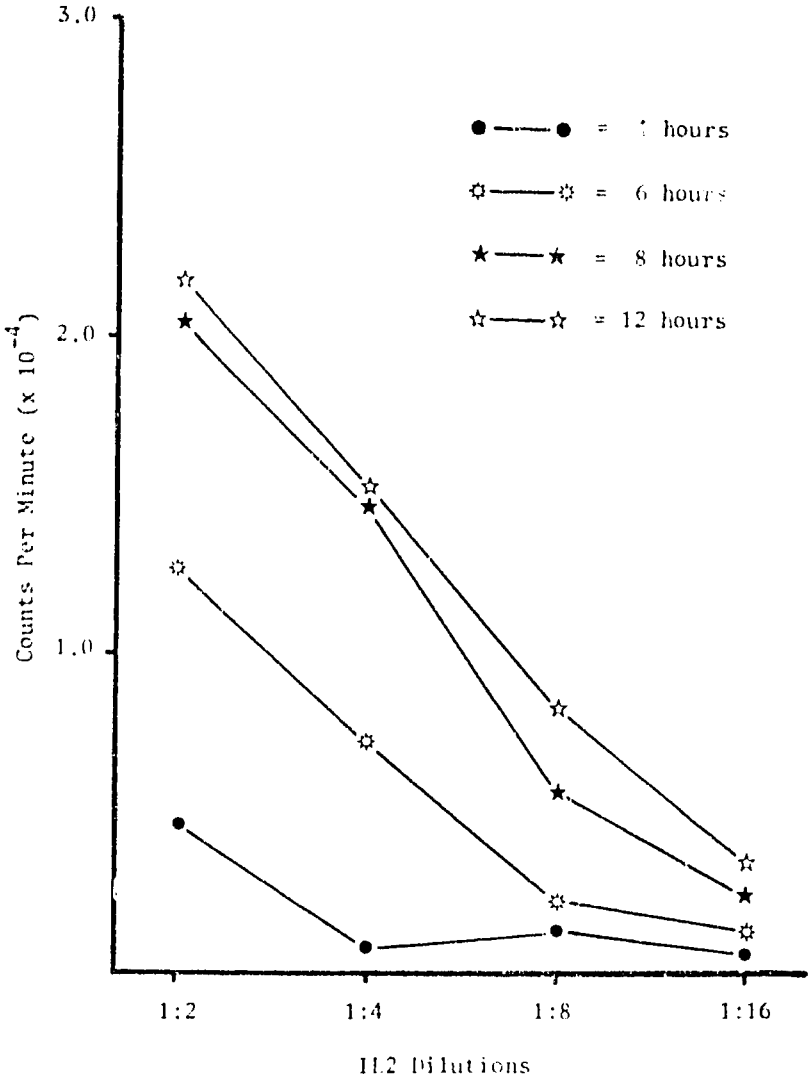


Figure 22. Optimum incubation time for IL2 production (cont.)

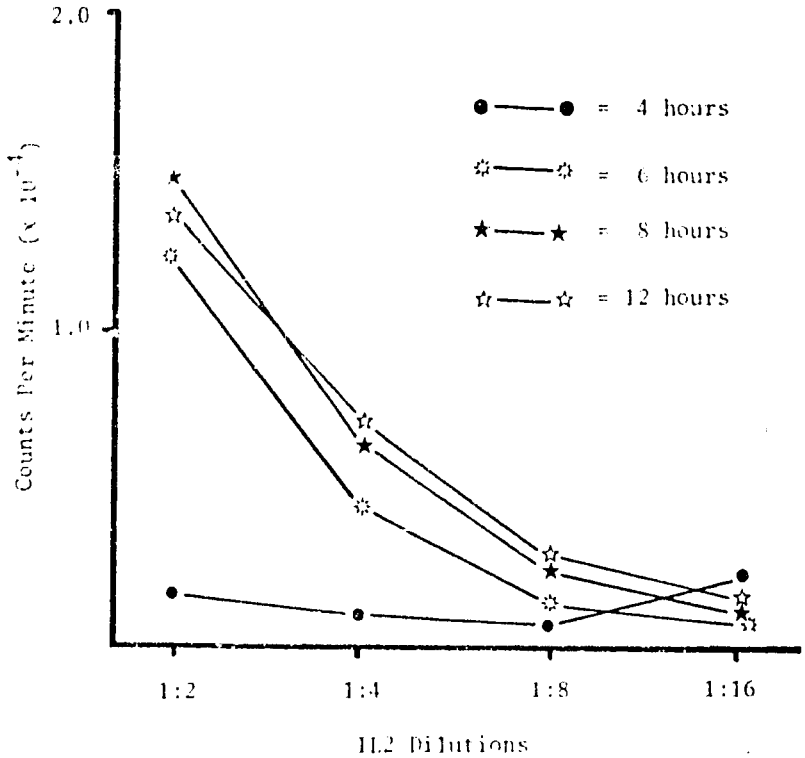


Figure 23. Optimum incubation time for IL2 production (cont.)

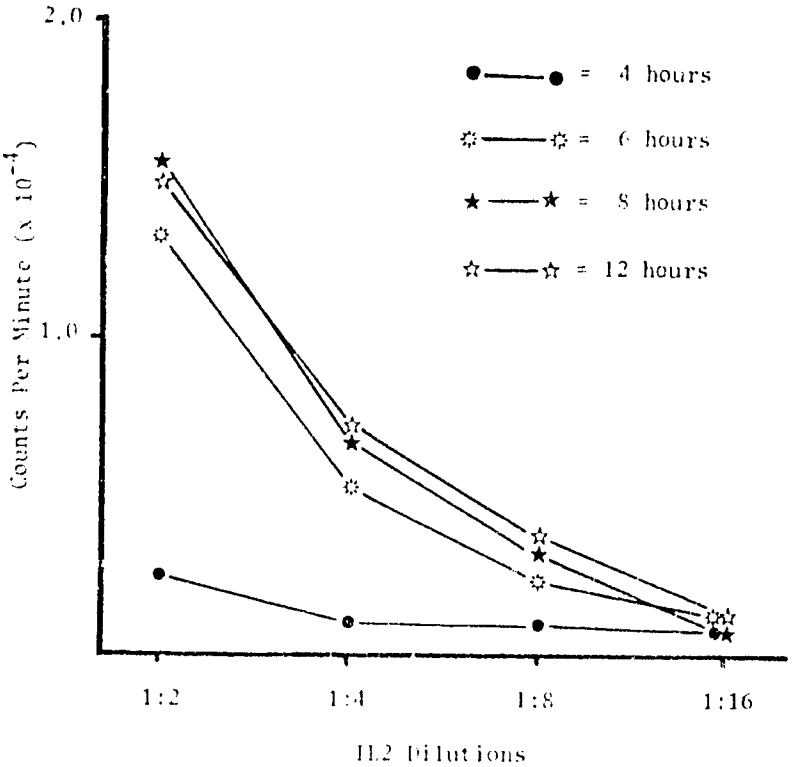


Figure 24. Optimum incubation time for IL2 production (cont.)

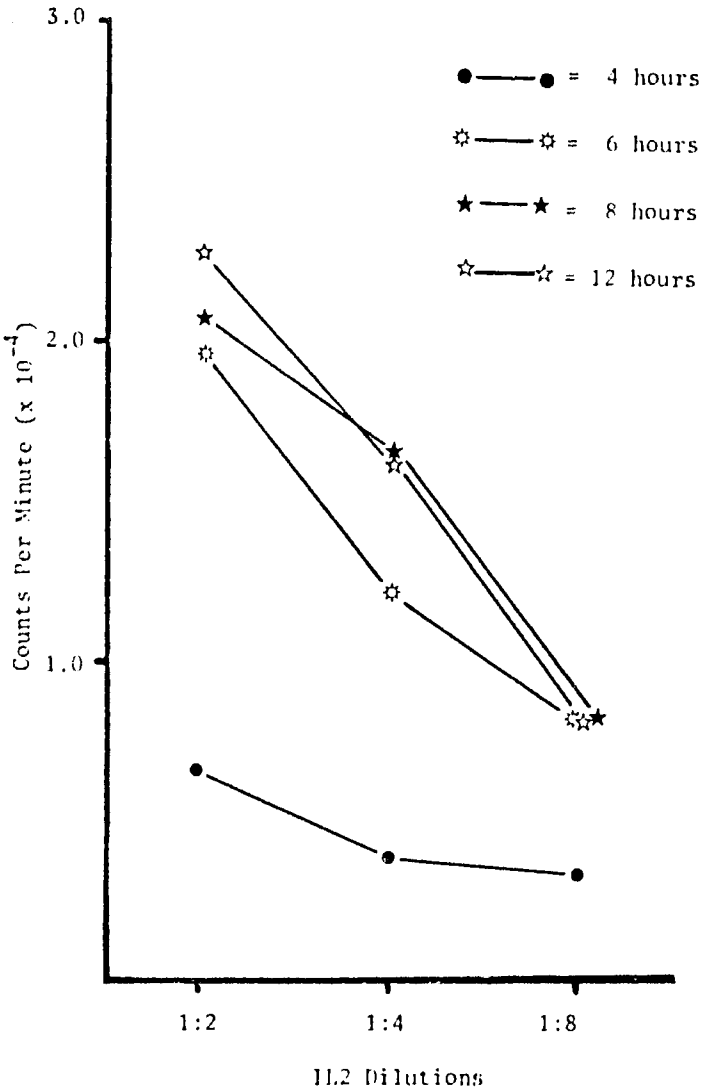


Figure 25. Optimum incubation time for IL2 production (cont.)

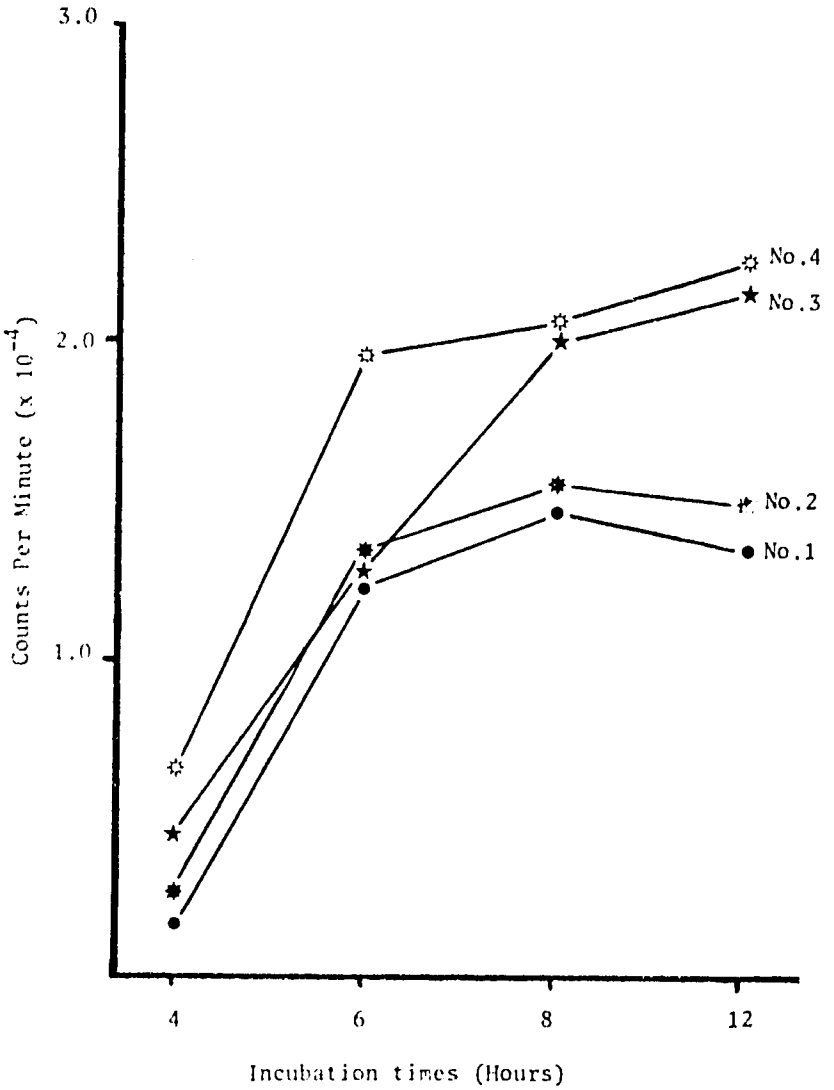


Figure 26. Optimum incubation time for IL2 production (tested at 1:2 dilution)

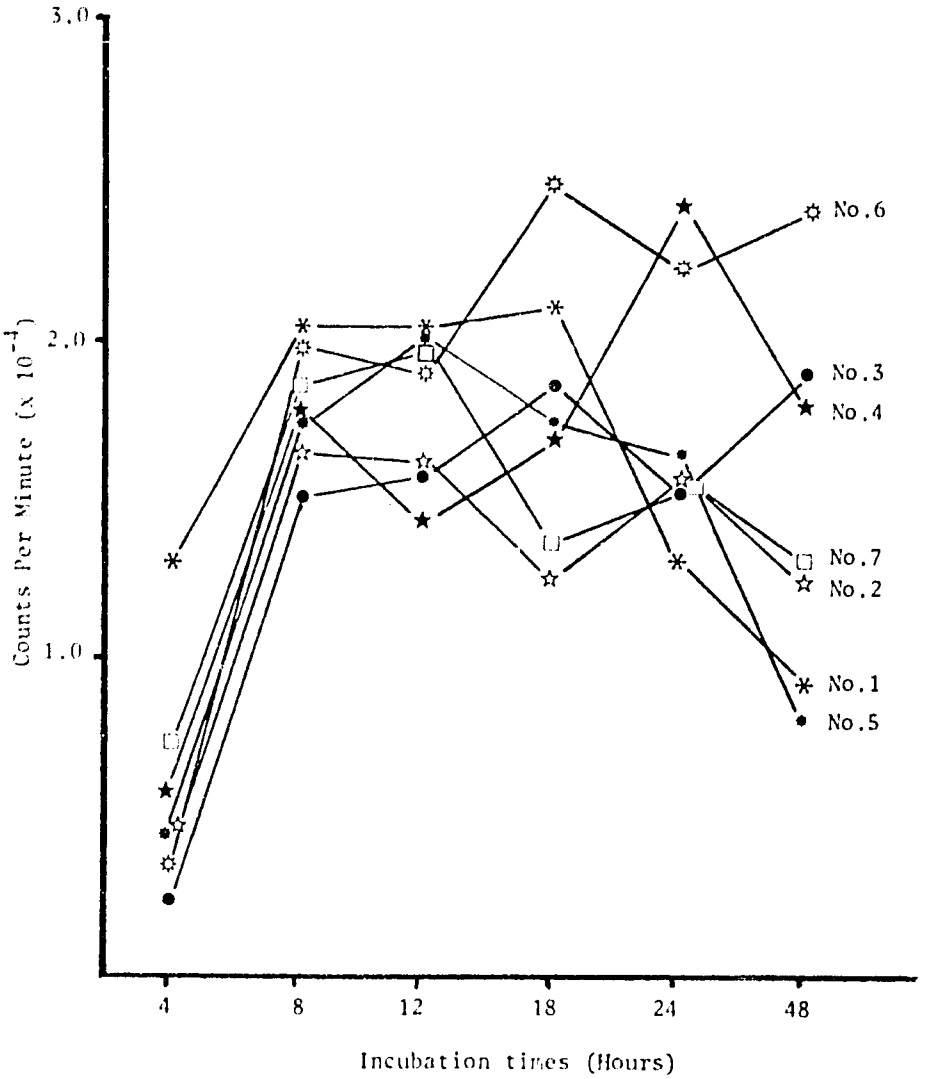


Figure 27. Optimum incubation time for IL2 production (tested at 1:2 dilution)

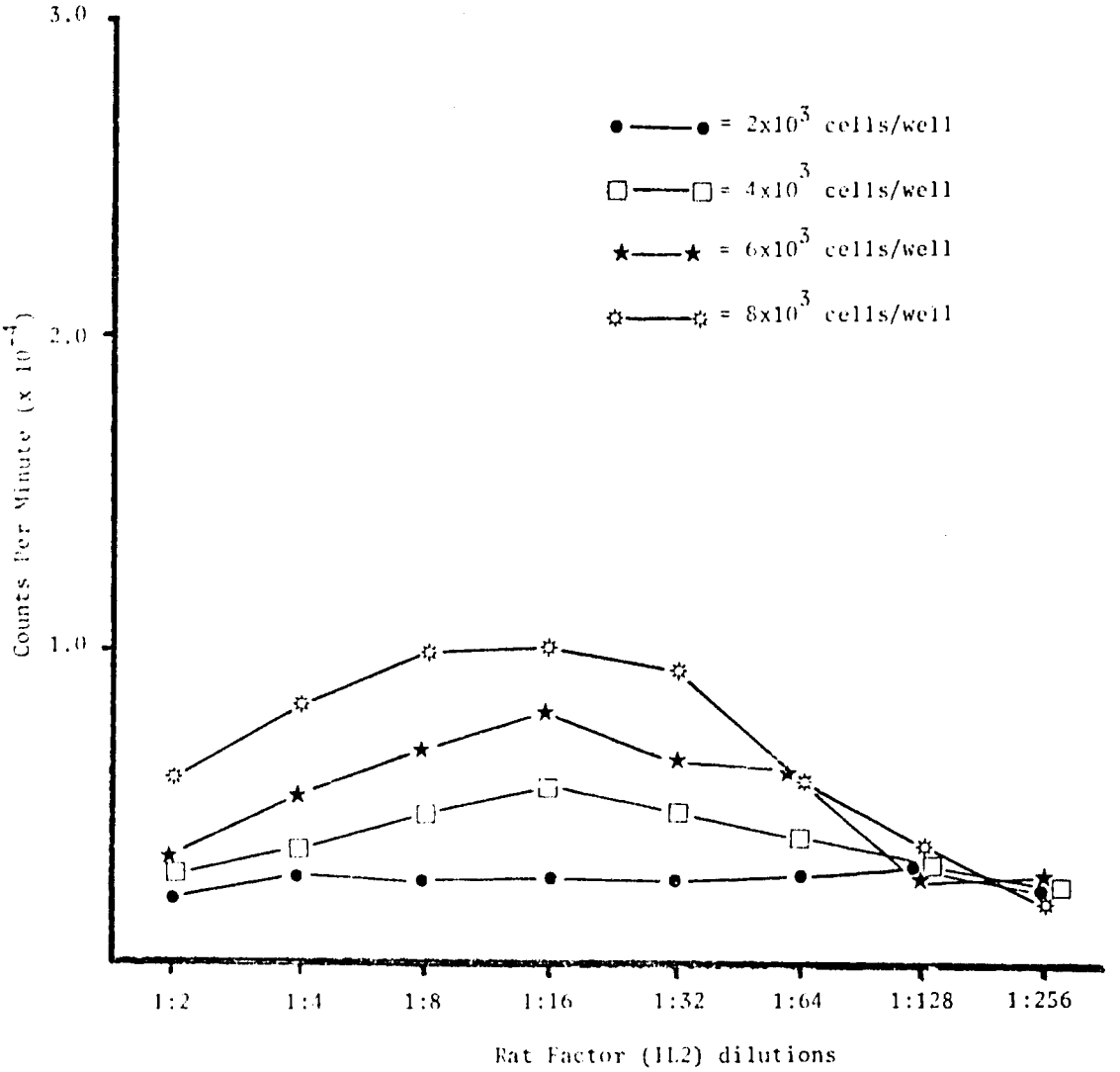


Figure 28. Optimum concentration of CTLL-2 for IL2 determination

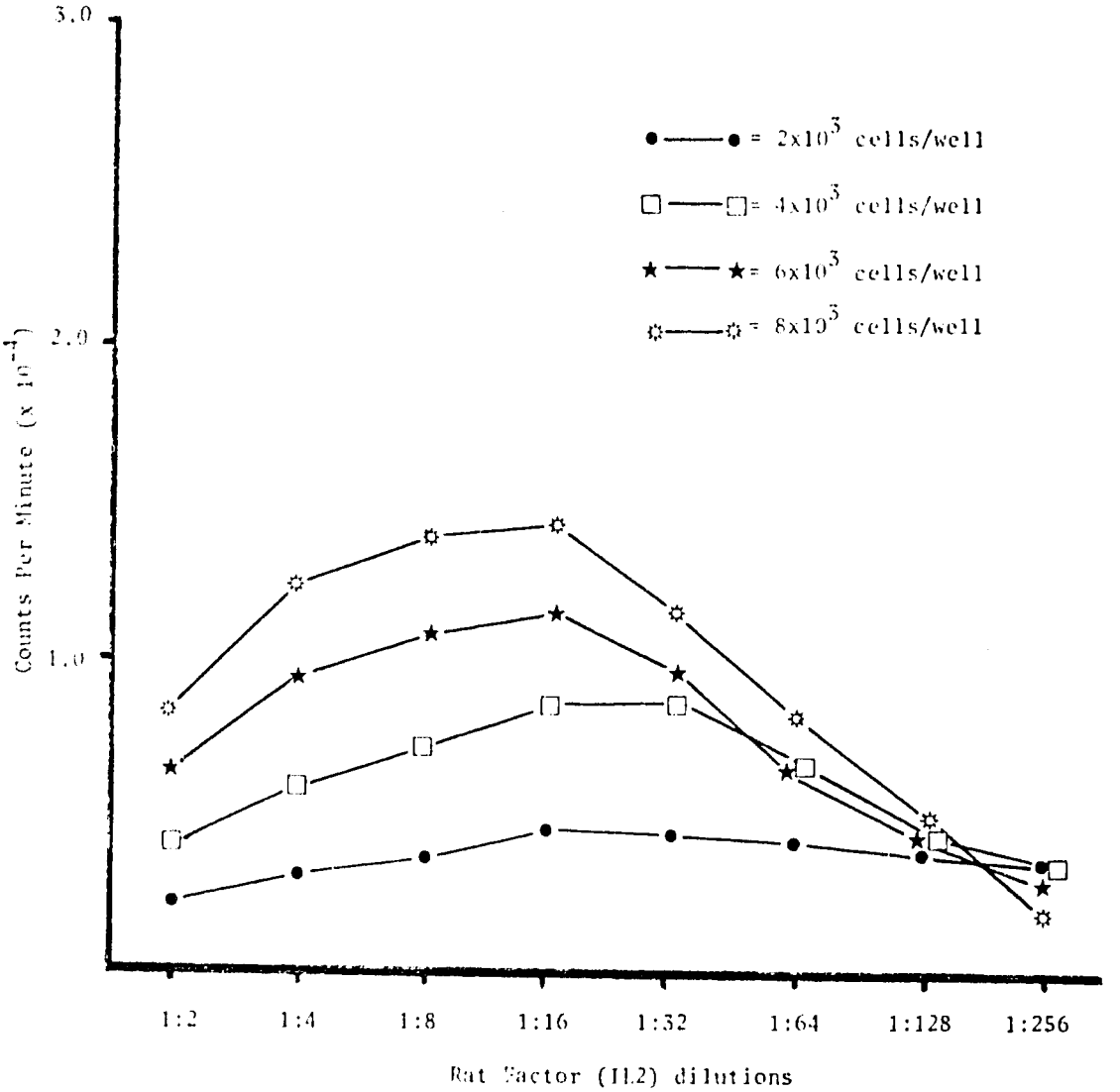


Figure 29. Optimum concentration of CTLL-2 for IL2 determination (cont.)

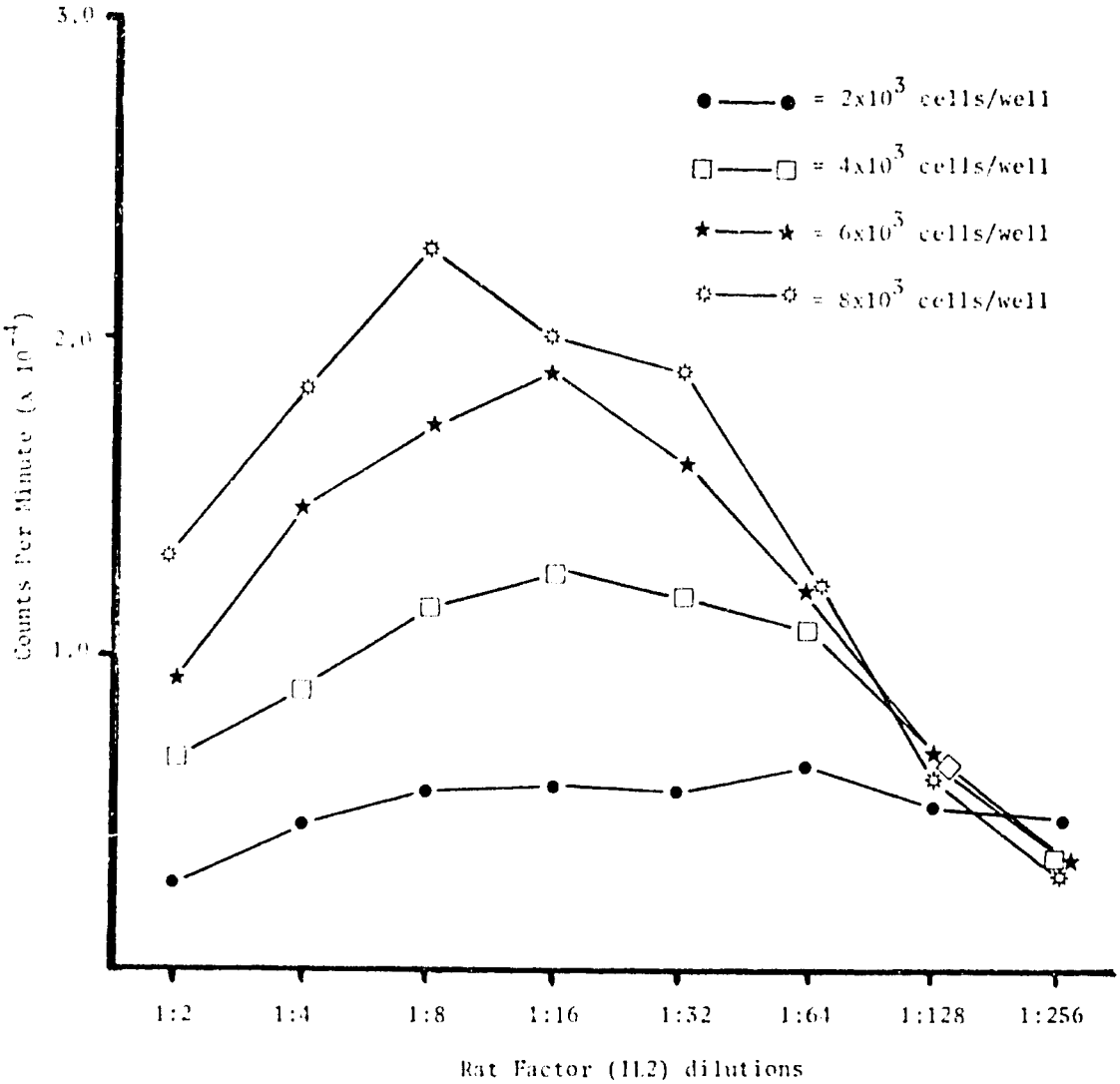


Figure 30. Optimum concentration of CTLL-2 for IL2 determination (cont.)

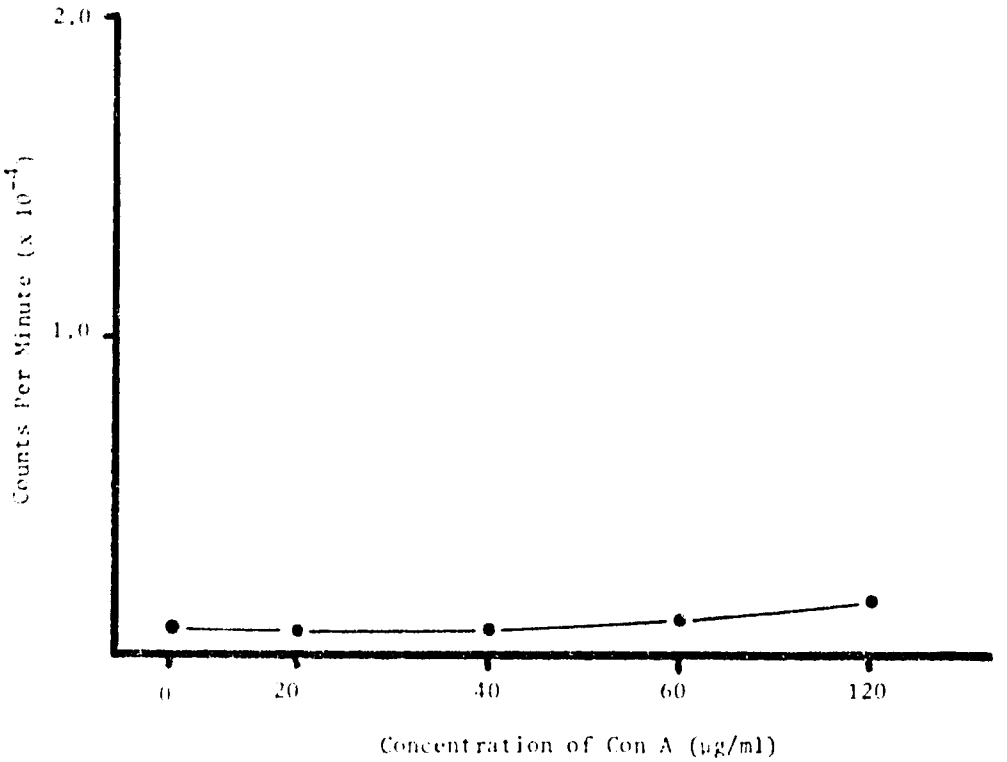


Figure 31. Effect of Con A to CTLL-2 cell line

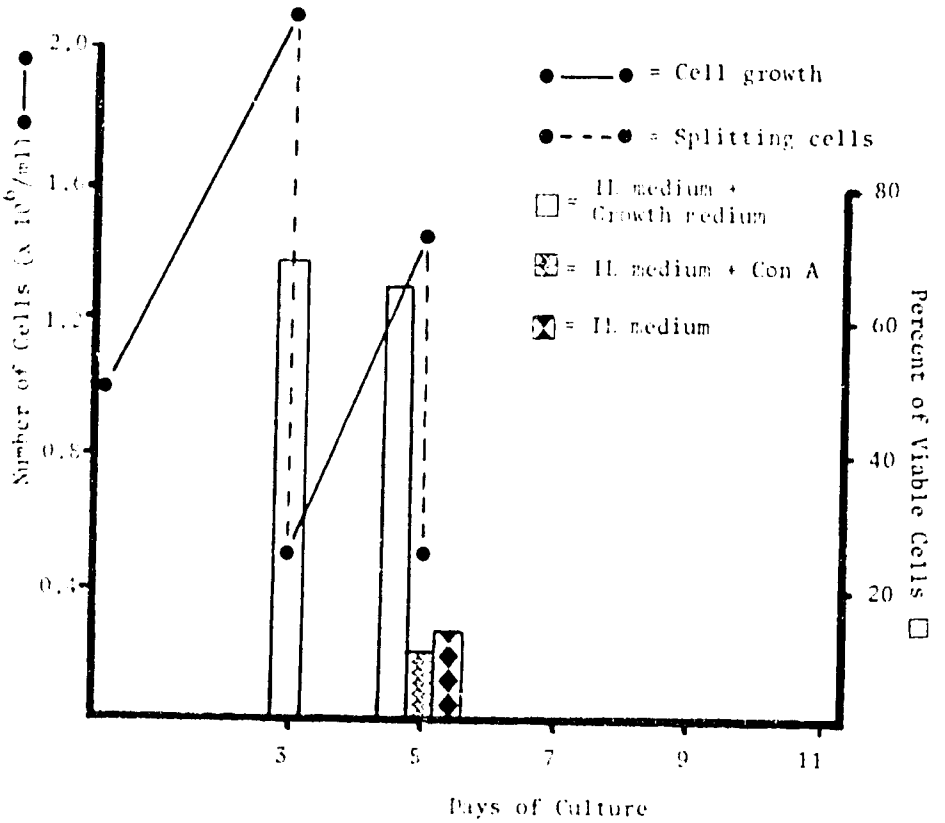


Figure 32. Production and isolation of mouse IL2-dependent spleen cells.

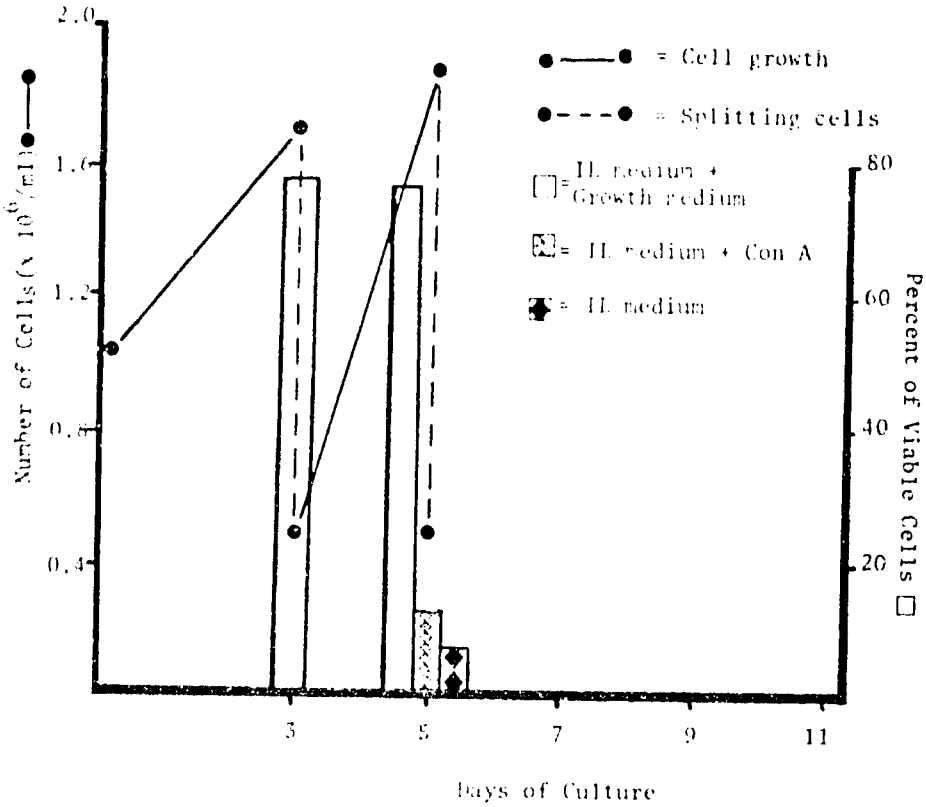


Figure 33. Production and isolation of mouse IL2-dependent spleen cells (cont.)

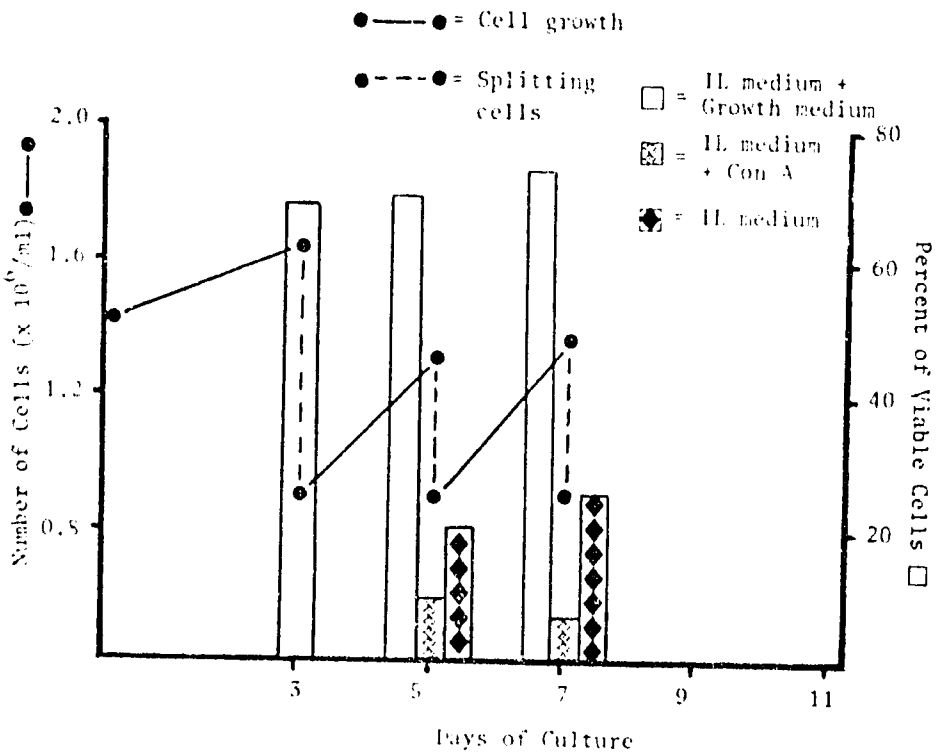


Figure 31. Production and isolation of mouse IL2-dependent spleen cells. (cont.)