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APPENDICES

A Head and Neck Cancer

B Physiological Characteristics of the Patients and Biochemical Laboratory Results

C Solvent Preparations

D Determination of Serum Methotrexate Concentration by Canfell, Chen, Cohen, Collier, Howell, Lawson, and Watson

E Paired T-Test

F Standard Curve Determination

G Semilogarithm Plots of Serum Methotrexate Level of 11 Patients

H Pharmacokinetic Analysis by Using the PCNONLIN Nonlinear Estimation Program

I Clinical Response of Patients: Diagnosis, Tumor Size and Tumor Response

APPENDIX A

Head and Neck Cancer

Cancer of the head and neck represents a varied group of tumors with different recurrence patterns, response rates and survival (Hancock, and Bradshaw, 1981). For detailed consideration, the region can be subdivided conveniently into areas and organs, as follows:

1. Lip
2. Mouth
    - 2.1 Tongue
    - 2.2 Floor of mouth
    - 2.3 Buccal and gingival mucosa
    - 2.4 Mandible and maxilla
    - 2.5 Palate
3. Tonsillar area and oropharynx
4. Postnasal space
5. Nasal cavity and paranasal sinuses
6. Larynx and hypopharynx
    - 6.1 Larynx
    - 6.2 Hypopharynx
7. Eye and orbit
    - 7.1 Eyelids
    - 7.2 Eye
    - 7.3 Orbital cavity
8. Salivary glands

9. Thyroid and parathyroids

10. Middle ear cleft

The estimated number of new head and neck cancer cases (excluding skin cancer) in 1973 at Siriraj Hospital was approximately 802 cases, this represented about 26% of the total new cancer cases. The ratio of male to female was approximately 1.4/1 (Faculty of Medicine, Siriraj Hospital, 1973). The usual time of diagnosis was over the age of 40, except for salivary gland and nasopharyngeal tumors, which might occur in younger age groups. A common etiologic factor (i.e., cigarette smoking) has resulted in a large increase of lung cancer (Million, Cassisi, and Wittes, 1985).

APPENDIX B

Physiological Characteristics and Biochemical Laboratory Results of the Patients

Table 13. Physiological characteristics of the patients.

Patient numbers	Sexs	Ages (years)	Weights (kg)
1	M	25	50
2	F	56	50
3	M	63	48
4	M	61	56
5	F	65	35
6	M	70	55
7	F	22	50
8	M	60	60
9	F	64	46
10	M	63	63
11	F	57	38
	Mean±SD	55.09±16.09	50.09±8.48

M = Male, F = Female

Table 14. Biochemical laboratory results.

Test	Normal value	Results										
		Patient										
		1	2	3	4	5	6	7	8	9	10	11
BS	60-100 mg/dl	85	125	110	320	120	210	80	105	100	90	100
BUN	8-20 mg/dl	9	11	11	18	5	10	7	10	11	19	8
Cr	0.7-1.5 mg/dl	0.9	0.8	0.8	1.6	1	1.3	0.8	1	0.9	1	0.5
UA	3-7.5 mg/dl	9	2.9	6.4	3.9	3.6	5.6	4	4.3	4.7	6.1	2.2
TP	6.5-7.5 g/dl	7.6	7.6	6.6	6.9	6.7	5.9	6.9	7.6	6.8	7.9	6.7
Alb	4-5.5 ng/dl	4.6	4.4	4.5	4.3	3.5	3.7	4.4	4	4.4	4.2	3.5
T.Bili	0.2-1 mg/dl	0.4	0.8	0.6	0.8	0.4	0.2	2.2	0.4	0.2	0.6	0.8
D.Bili	0-0.2 mg/dl	0.1	0.2	0.1	0.3	0.1	0.1	0.3	0.1	0.1	0.2	0.1
Chol	150-280 mg/dl	249	193	211	218	270	218	145	213	224	208	180
SGOT	8-28 Units	20	24	17	29	17	21	19	13	16	25	20
AP	9-35 IU/L	22	36	38	40	28	33	16	27	41	27	35
Hct	37-54%	39	34	45	36	30	35	28	33.5	36.5	37	34
Hb	14-16 g/dl	-	11	14.5	12.5	9.7	11.6	8.9	11	12	12	11
WBC	4500-11000/mm^2	8200	9600	8200	5700	5300	7800	7265	6000	6950	6200	4200
D/C Ne	40-60%	72	65	78	70	63	43	57	66	62	76	77
E	1-3%	6	3	2	1	1	27	9	7	5	1	-
B	0-1%	-	-	-	-	-	-	-	-	1	-	-
Ly	20-40%	21	30	17	29	36	27	31	26	30	20	19
Mo	4-8%	1	2	3	-	-	3	3	1	2	3	4

APPENDIX C

Solvent Preparations

1. 0.025 M Phosphate Buffer pH 6.25

0.025 M Na_2HPO_4 solution and 0.025 M KH_2PO_4 solution were mixed to adjust pH of the solution to be 6.25.

2. 0.5 M Phosphate Buffer pH 6.6

0.5 M Na_2HPO_4 solution and 0.5 M KH_2PO_4 were mixed to adjust pH of the solution to be 6.6.

3. 0.15 M Sodium Acetate pH 4.6

Adding acetic acid to 0.15 M sodium acetate solution to adjust pH of the solution to be 4.6.

4. 0.005 M Hexanesulfonic Acid pH 3.75

Adding acetic acid to 0.005 M hexanesulfonic acid to adjust pH of the solution to be 3.75.

5. 0.01 M KH_2PO_4 pH 4.5

1.36 g of KH_2PO_4 was dissolved in 1000 ml of distilled water. Its pH was 4.5.

6. 0.1 M Tris-NaH_2PO_4 pH 6.7

0.1 M Tris (hydroxymethyl) aminomethane solution and 0.1 M NaH_2PO_4 solution were mixed to adjust pH of the solution to be 6.7.

7. 0.25 M Phosphate Buffer pH 6.8

0.25 M Na_2HPO_4 solution and 0.25 M KH_2PO_4 solution were mixed to adjust pH of the solution to be 6.8.

8. 0.1 M Phosphate Buffer pH 6.8

0.1 M Na_2HPO_4 solution and 0.1 M KH_2PO_4 solution were mixed to adjust pH of the solution to be 6.8.

9. 0.2 M Acetate Buffer pH 5.0

Adding ammonia water to 0.2 M acetic acid solution to adjust pH of the solution to be 5.0.

APPENDIX D

Determination of Serum Methotrexate Concentration by Canfell, Chen, Cohen, Collier, Howell, Lawson, and Watson

1. Determination of Serum Methotrexate Concentration by Canfell.

column : μ Bondapak/phenyl (0.39 X 25 cm)

detector : ultraviolet spectrophotometer, at 303 nm

flow rate : 2 ml/min

solvent : 0.15 M sodium acetate buffer pH 4.6/ acetonitrile = 89/11

extraction: serum 1 ml + acetonitrile 1 ml

↓ centrifuged

the supernatant was extracted with anh. ethyl ether 5.5 ml and n-butanol 2.9 ml for 2 mins

↓ centrifuged

the organic layer was discarded

↓

the water phase + anh. ethyl ether 2.5 ml

↓ shaked ↓ centrifuged

the water phase was injected into the column

2. Determination of Serum Methotrexate Concentration by Chen.

column : partisil PXS 10/25 SCX (0.46 X 25 cm)

detector : ultraviolet spectrophotometer, at 313 nm

flow rate : 2 ml/min

solvent : 0.02 M $(NH_4)H_2PO_4$ with 0.2% H_3PO_4 / acetonitrile = 90/10

extraction: serum 0.2 ml + acetonitrile 0.5 ml

↓ centrifuged

the supernatant + ethyl acetate 1 ml

+ isoamyl alcohol 100 µl

↓ shaked, centrifuged

the water phase was injected into HPLC

3. Determination of Serum Methotrexate Concentration by Cohen.

column : RP-8 (0.41 X 25 cm)

detector : ultraviolet spectrophotometer, at 313 nm

flow rate : 1.5 ml/min

solvent : 0.1 M phosphate buffer pH 6.8/MeOH = 85/15

IS : p-aminoacetophenone

extraction: serum 1 ml + IS 0.25 mcg + 1N $HClO_4$ 1.5 ml

↓ centrifuged

the supernatant + solid $(NH_4)_2SO_4$ 5 g

+ ethyl acetate/isopropanol (10/1) 2 ml

↓ shaked for 20 mins

the organic layer was evaporated to dryness

at 60°C under a stream of nitrogen

↓

residue was reconstituted in 0.005 M

K_2HPO_4 100 µl

↓

the solution was injected into the column

4. Determination of Serum Methotrexate Concentration by Collier.

column : radial µ Bondapak

detector : ultraviolet spectrophotometer, at 305 nm

folw rate : 0.8 ml/min

solvent : 0.01 M KH_2PO_4 pH 4.5/acetonitrile = 85/15

IS : 8-chlorotheophylline

extraction: Sep pak was washed with MeOH 10 ml and 10 ml of 0.2 M acetate buffer pH 5.0

↓

serum 0.3 ml + IS 6.25 mcg + acetate buffer 5 ml

↓

the solution was applied to Sep pak

↓

Sep pak was washed with water 10 ml

↓

MTX and IS were eluted with MeOH 2 ml

↓

the eluent was evaporated to dryness at 60°C under nitrogen gas

↓

the residue was reconstituted in 0.005 M HCl 200 µl

↓

↓ centrifuged

the solution was injected into the column

5. Determination of Plasma Methotrexate Concentration by Howell.

column : partisil PXS 10/25 ODS (0.46 X 25 cm)

detector : ultraviolet spectrophotometer, at 280 or 305 nm

flow rate : 1 ml/min

solvent : 0.005 M 1-hexanesulfonic acid pH 3.75/MeOH = 70/30

extraction: plasma 1 ml + 6% $HClO_4$ 1 ml

↓

the supernatant was neutralized with 1 M KOH

↓ centrifuged

the supernatant was extractred with ethyl acetate/isopropanol (10/1)

↓

the organic layer was evaporated under nitrogen

↓

the residue was dissolved in water 100 μl

↓

the solution was injected into the column

6. Determination of Serum Methotrexate Concentration by Lawson.

column : hypersil-ODS (0.4 X 12 cm)

detector : ultraviolet spectrophotometer, at 305 nm

flow rate : 1 ml/min

solvent : 0.1 M tris-NaH_2PO_4 pH 6.7/MeOH =80/20

IS : N-[4[[2,4diamino-6-quinazolinyl)methylamino] benzoyl]]aspatic acid

extraction: serum 0.5 ml +IS 0.3 ml (10 mcg/ml in 2 M $HClO_4$)

↓ centrifuged

the supernatant was injected into the column

7. Determination of Plasma Methotrexate Concentration by Watson.

column : partisil 10-SAX (0.46 X 25 cm)

detector : ultraviolet spectrophotometer, at 315 nm

flow rate : 1.2 ml/min

solvent : 0.025 M sodium phosphate buffer pH 7.0

IS : N-[4[[2,4-diamino-6-quinazolinyl)methylamino] benzoyl]]aspartic acid

extraction: plasma 1 ml + IS 4 mcg + water 1 ml

+ 2 N $HClO_4$ 1.5 ml

↓ centrifuged

the supernatant was added with

solid $(NH_4)_2SO_4$ 5 g and extracted by

ethyl acetate/isopropanol (10/1) 2 ml

↓

the organic layer was evaporated under nitrogen

↓

the residue was reconstituted in water 100 µl

↓

the solution was injected into the column

APPENDIX E

Paired T-Test

Analyzed serum methotrexate concentrations obtained from applying methotrexate through washed Sep pak were compared with methotrexate standards by using paired t-test

Table 15. Calculation of the difference between methotrexate standard and analyzed methotrexate concentration obtained from washed Sep pak.

MTX Standards (mcg/ml)	Analyzed MTX concentrations (mcg/ml)	d_i	d_i^2
2.652	2.6952	-0.0432	0.00187
4.420	4.3847	0.0353	0.00125
6.188	6.1138	0.0742	0.00551
		0.0663	0.00863

$H_0 : Ud = 0$

$H_a : Ud \neq 0$

$$\bar{d} = \frac{\sum d_i}{n} = \frac{0.0663}{3} = 0.0221$$

$$Sd^2 = \frac{n\sum d_i^2 - \left(\sum d_i\right)^2}{n\,(n-1)}$$

$$= \frac{3 \times 0.00863 - (0.0663)^2}{3\,(3-1)}$$

$$= 0.00358$$

$$Sd = 0.0598$$

$$S_{\bar{d}} = \frac{Sd}{n} = \frac{0.0598}{3} = 0.0345$$

$$t = (\bar{d} - Ud)/S_{\bar{d}}$$

$$t = \frac{0.0221-0}{0.0345}$$

$$t = 0.6406$$

Percentile of t Distributions

d.f. $t_{.90} = 1.886.$

2

So H_0 was accepted.

APPENDIX F

Standard Curve Determination

Table 16. Typical standard curve data for methotrexate concentrations in human serum estimated using linear regression.[1]

MTX concentration (mcg/ml)	Peak height ratio of MTX/8-CT	Inversely[2] estimated concentrations (mcg/ml)	% Theory[3]
0.1768	0.0582	0.1938	109.62
0.442	0.1509	0.4932	111.58
0.884	0.2444	0.7952	89.95
2.652	0.7521	2.4351	91.82
4.420	1.3479	4.3595	98.63
6.188	1.8857	6.0966	98.52
8.840	2.7429	8.8653	100.29
		Mean	100.06
		SD	8.15
		CV[4]	8.15

1. $r^2 = 0.9952$, A = -0.0018, B = 0.3096 (y = A + Bx)
2. Inversely estimated concentration = (peak height ratio + 0.0018)/ 0.3096
3. % Theory = Inversely estimated concentration/ methotrexate concentration
4. Coefficient of Variation (CV) = (SD X 100)/Mean

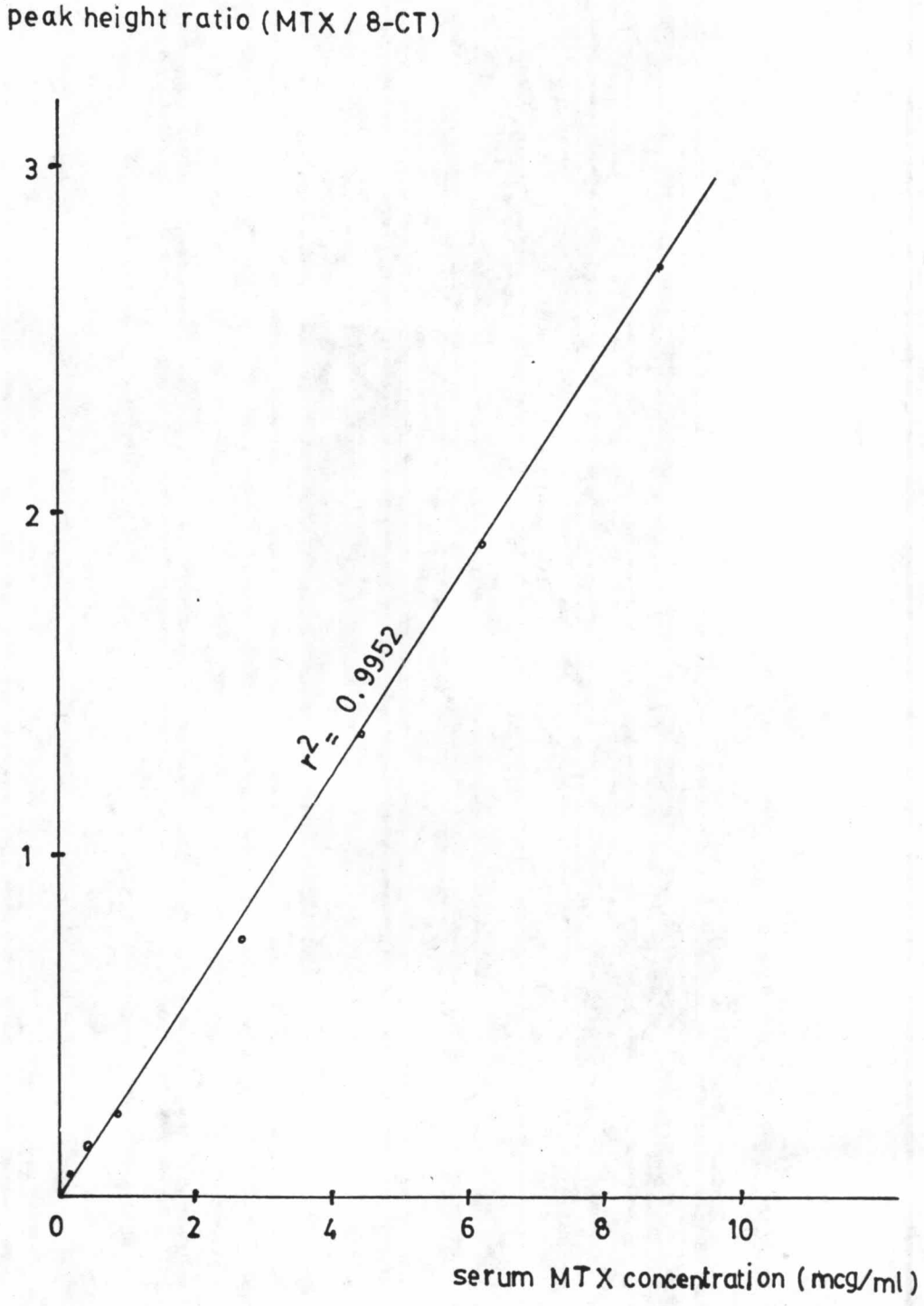

Figure 29. Typical standard curve for methotrexate concentration in human serum.

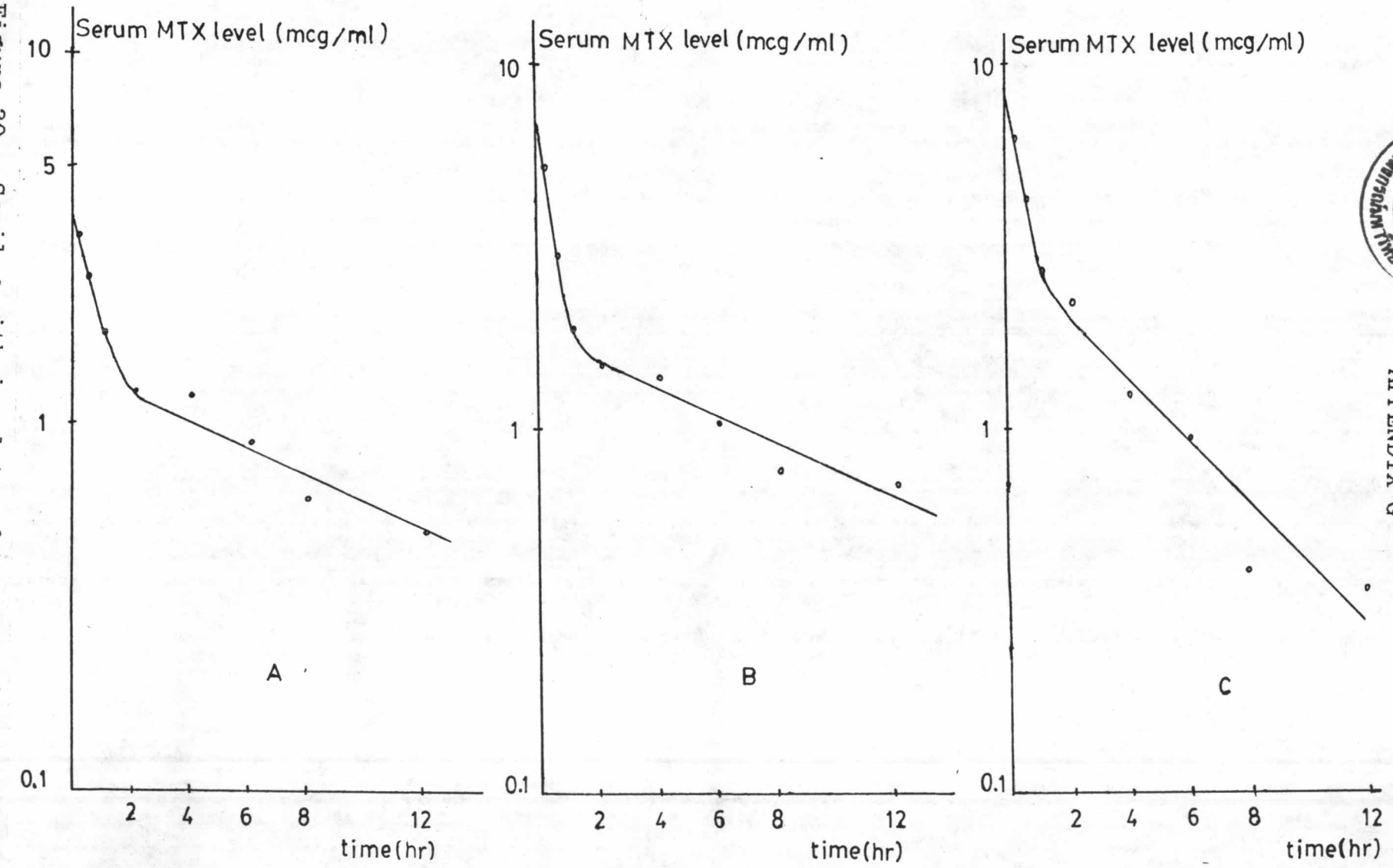

Figure 30. Semilogarithmic plots of serum methotrexate level of 11 patients.

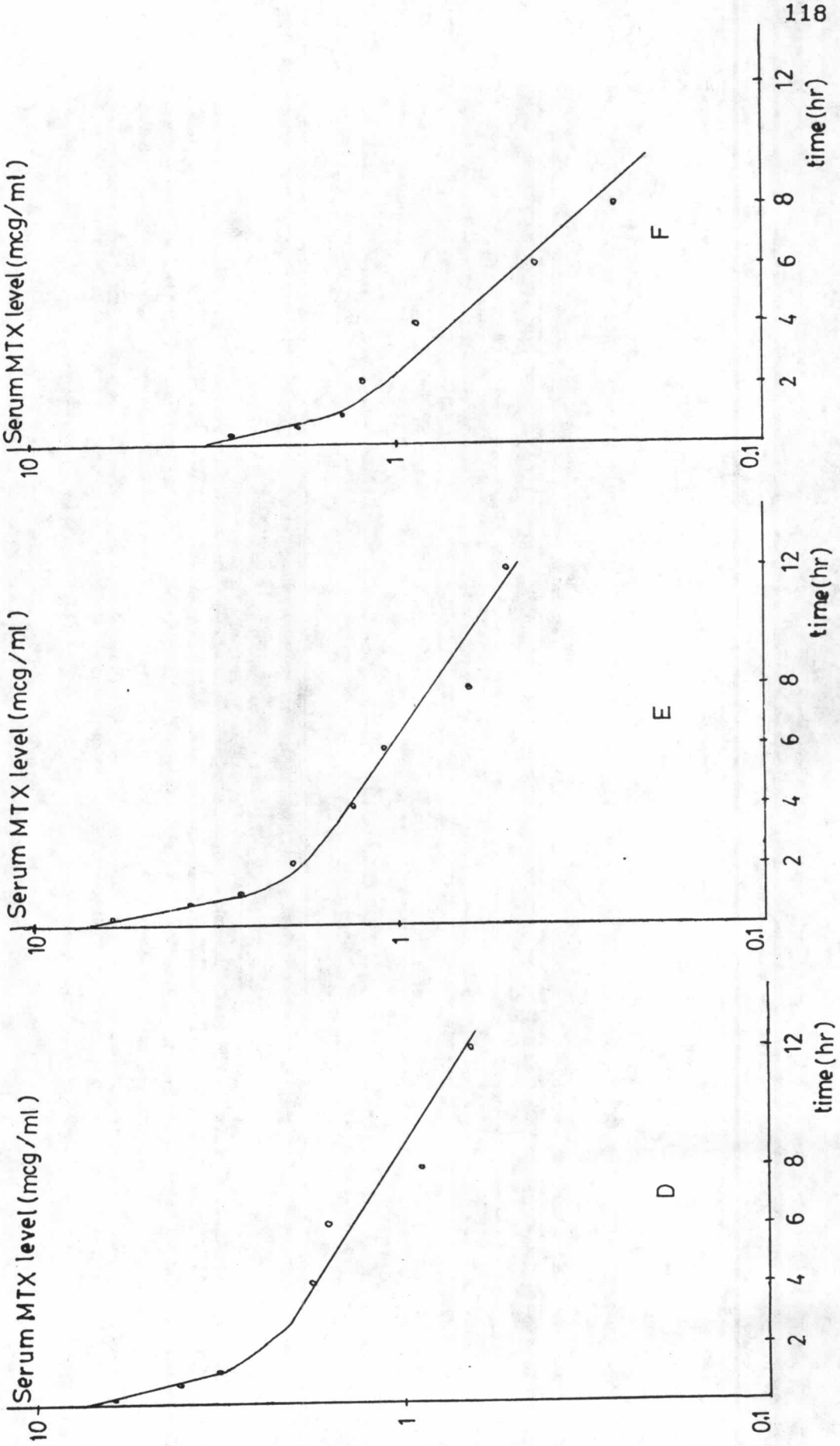

Figure 30 (cont.). Semilogarithmic plots of serum methotrexate level of 11 patients.

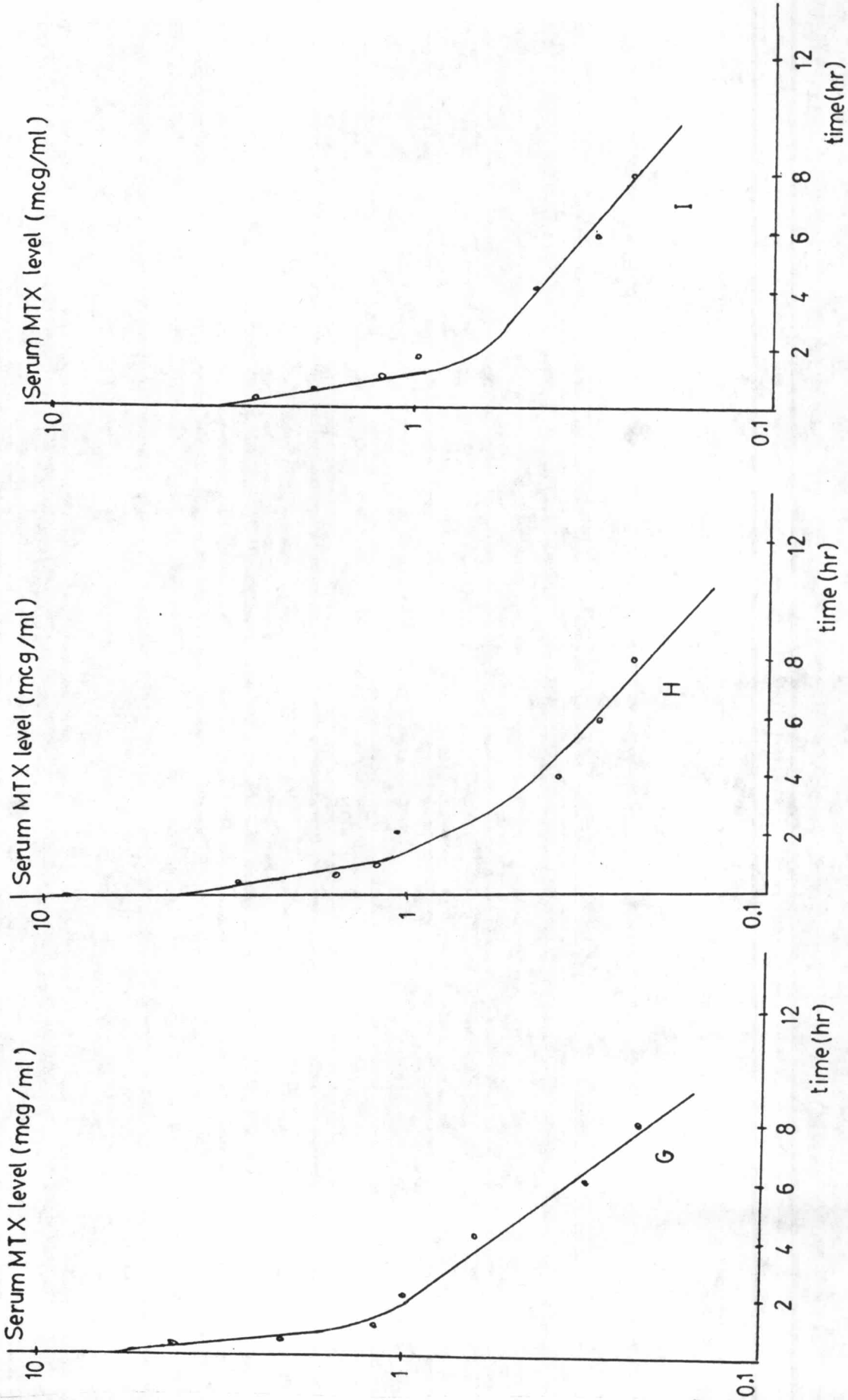

Figure 30 (cont.). Semilogarithmic plots of serum methotrexate level of 11 patients.

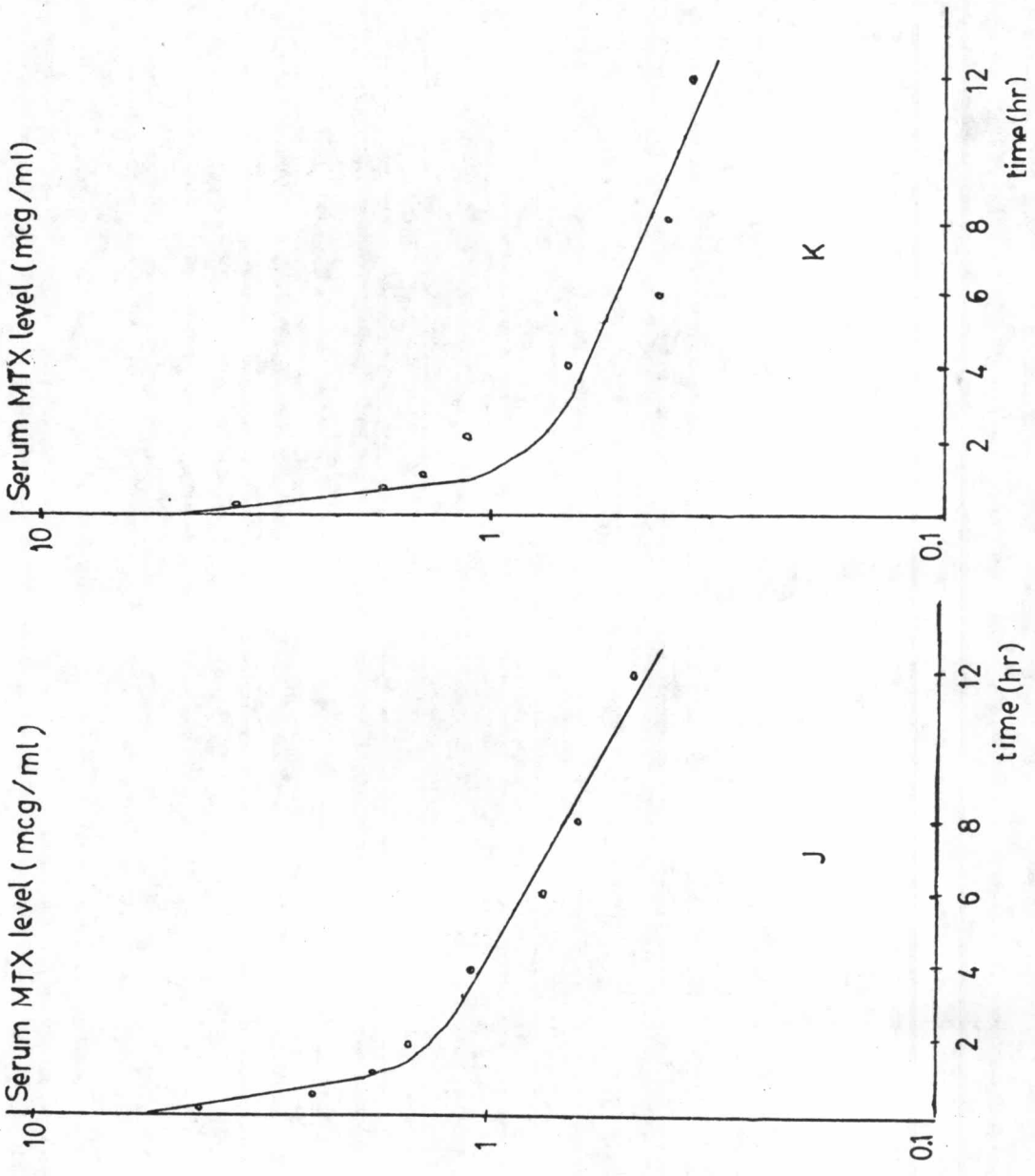

Figure 30 (cont.). Semilogarithmic plots of serum methotrexate level of 11 patients.

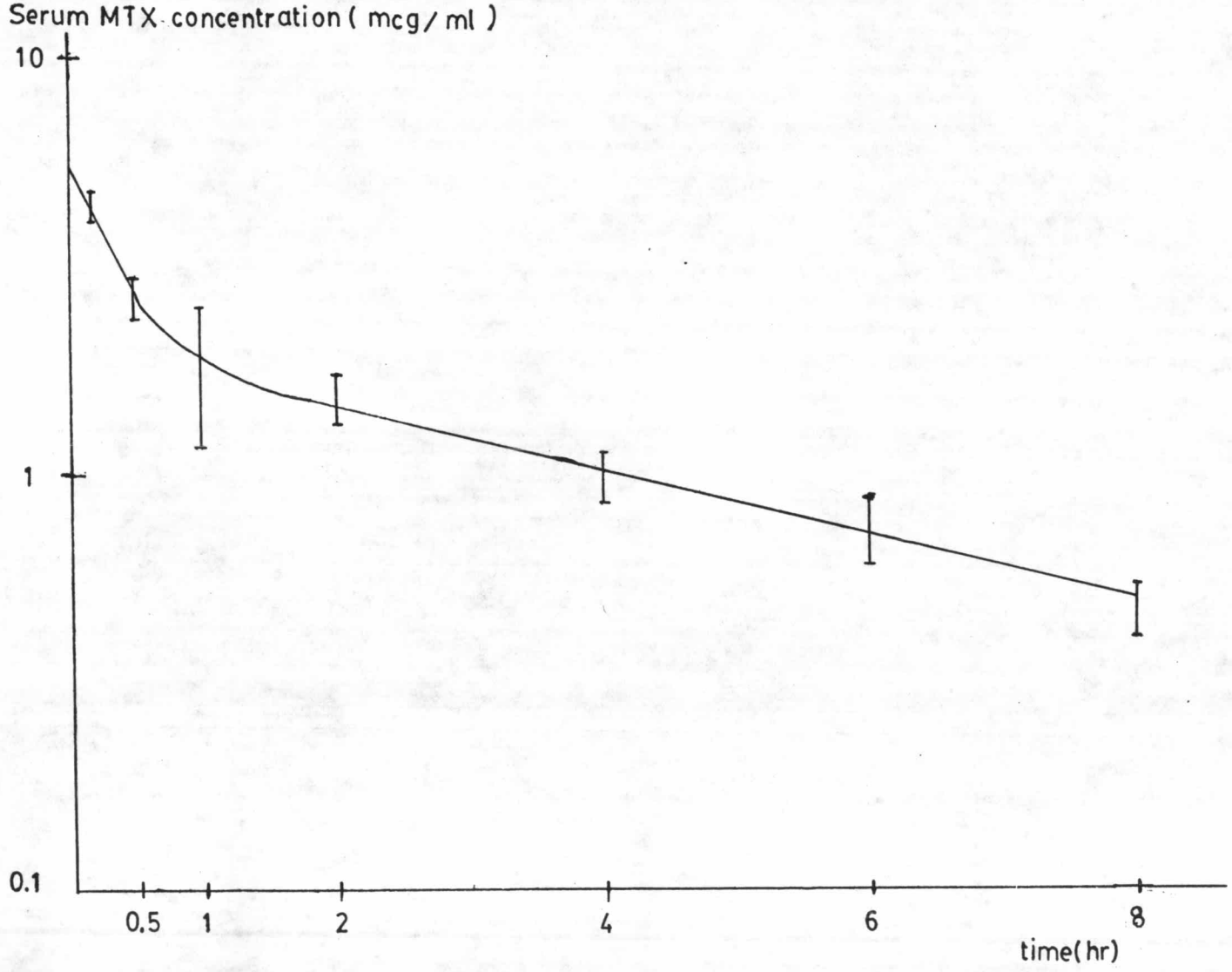

Figure 31. Average serum methotrexate profile from 11 patients.

APPENDIX H

Pharmacokinetic Analysis by Using the PCNONLIN Nonlinear Estimation Program

It was proposed that the time course of serum methotrexate for each patient could be well described by a two-compartment model with bolus input and first-order output (Model 8).

The initial estimates of the parameters (A, B, α, β) used with PCNONLIN nonlinear estimation program were obtained by graphic procedure using the method of residuals (Gibaldi, et al., 1982b).

For example, the data set from Table 9 in patient number 5 was chosen. We plotted C_t versus t on a semilogarithmic graph paper and use the method of residuals to determine A, B, α, β (see Figure 30E and Table 17). The intercepts on the y axis after extrapolation of the residual and terminal lines for distribution and elimination were 5.62 and 2.75 mcg/ml, respectively.

The slope of the terminal portion of the curve was calculated as follows:

$$\beta = \frac{\ln 0.86 - \ln 0.64}{1.8} = 0.1641 \text{ hr}^{-1}$$

as well as the

$$\alpha = \frac{\ln 3.1 - \ln 0.086}{1.2} = 2.9873 \text{ hr}^{-1}$$

The final estimation of the parameters was obtained by repeated entering the computed parameter values as initial estimation until the values were stabilized. Results obtained from the computer analysis of the estimated pharmacokinetic parameters were shown in Figure 33 and Table 10.

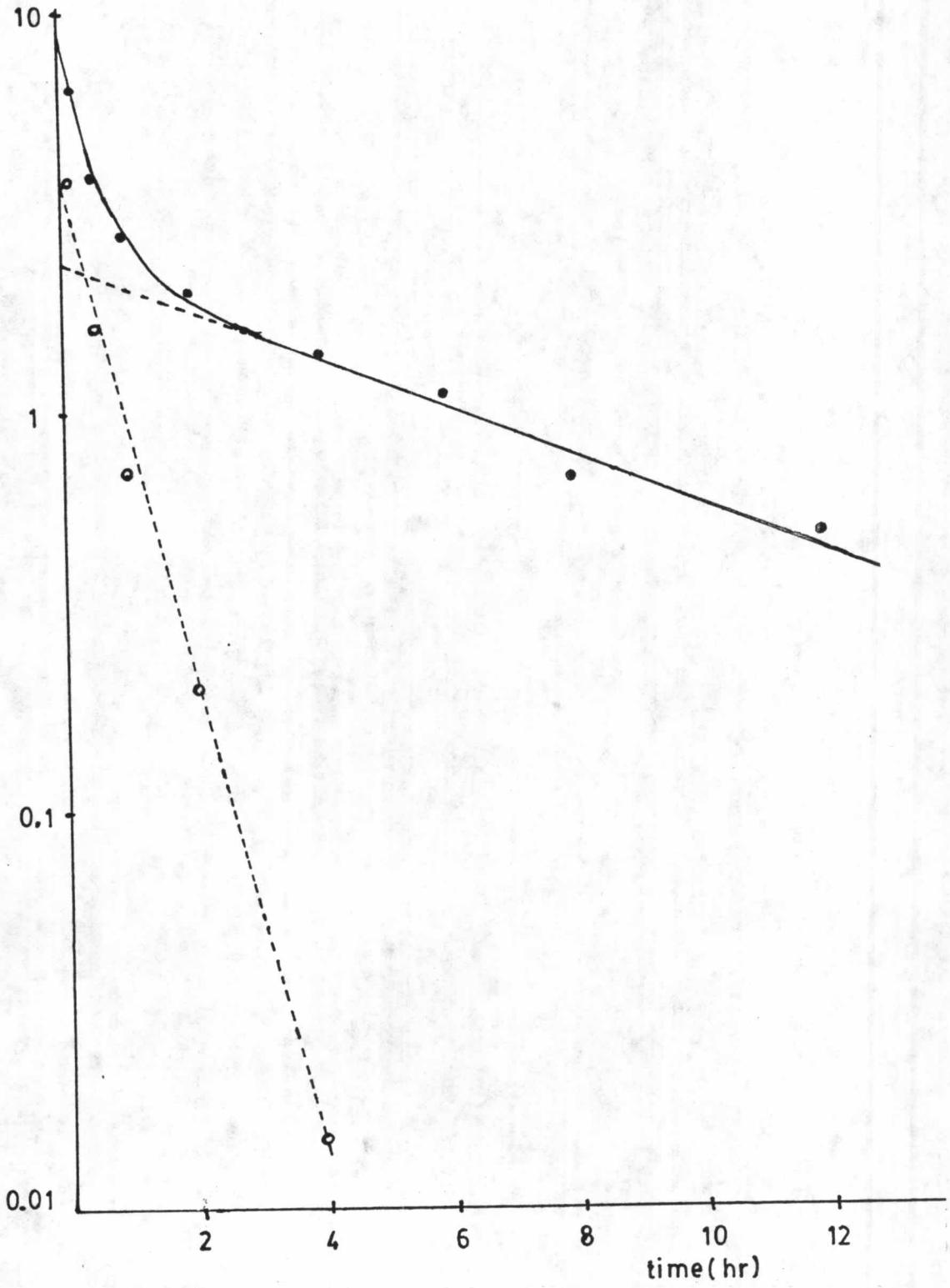

Figure 32. Graphical technique of calculating estimated pharmacokinetic parameters in the serum methotrexate concentration-time curve by the method of residuals.

Table 17. Striping biexponentials from set of the serum methotrexate concentration in patients no. 5.

Time (hr)	C_{obs} (mcg/ml)	$\hat{C}_t = 2.75e^{-0.1641t}$	$R_1 = C_{obs} - \hat{C}_t$	$\hat{R}_1 = 5.62e^{-2.9873t}$	$C_{pred} = \hat{C}_t + \hat{R}_1$	$\frac{C_{pred}}{C_{obs}} \times 100$
0.167	6.1029	2.6757	3.4272	3.4125	6.0882	99.76
0.5	3.7481	2.5334	1.2084	1.2620	3.7954	101.26
1	2.7096	2.3338	0.3758	0.2834	2.6172	96.59
2	1.9665	1.9806	-	0.0143	1.9949	101.44
4	1.3548	1.4265	-	0	1.4265	105.29
6	1.0980	1.0274	-	0	1.0274	93.57
8	0.6488	0.7399	-	0	0.7399	114.04
12	0.4887	0.3838	-	0	0.3838	78.53
					Mean	98.81
					SD	10.22
					CV	10.34

```
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LISTING OF INPUT COMMANDS

MODEL 8,'NLIN.LIB'
MODEL 8
REMARK  TWO COMPARTMENT MODEL - BOLUS INPUT, FIRST ORDER OUTPUT
REMARK  DEFINED IN TERMS OF A,B,ALPHA,BETA
REMA
REMA   NO.    PARAMETER      CONSTANT       SECONDARY PARM.
REMA   ---    ---------      --------       ---------------
REMA    1        A             DOSE             AUC
REMA    2        B                           K10 HALF LIFE
REMA    3      ALPHA                         ALPHA HALF LIFE
REMA    4       BETA                          BETA HALF LIFE
REMA    5                                       K10
REMA    6                                       K12
REMA    7                                       K21
REMA    8                                     VOLUME
REMA*************************************************************
REMA            I--------------------I
REMA   BOLUS    I                    I
REMA    IV  --> I   COMPARTMENT  1   I  ---> K10
REMA            I                    I
REMA            I--------------------I
REMA                 I          I
REMA            K12  I          I K21
REMA                 I          I
REMA                 I          I
REMA            I--------------------I
REMA            I                    I
REMA            I   COMPARTMENT  2   I
REMA            I                    I
REMA            I--------------------I
REMA*************************************************************
COMM
NPARM 4
NCON 1
NSEC 8
PNAMES 'A', 'B', 'ALPHA', 'BETA'
SNAMES 'AUC', 'K10-HL', 'ALPHA-HL', 'BETA-HL', 'K10', &
       'K12', 'K21', 'VOLUME'
```

Figure 33. The output of example 5-fitting data to Model 8 (Two - compartment model with bolus input and first order output) of the PCNONLIN library.

```
END
TEMP
A=P(1)
B=P(2)
ALPHA=P(3)
BETA=P(4)
T=X
D=CON(1)
V=D/(A+B)
K21=(A*BETA + B*ALPHA)/(A+B)
K10=ALPHA*BETA/K21
K12=ALPHA+BETA-K21-K10
END
FUNC1
F=(A*DEXP(-ALPHA*T))+(B*DEXP(-BETA*T))
END
SECO
S(1)=(A/ALPHA)+(B/BETA)
S(2)=-DLOG(.5)/K10
S(3)=-DLOG(.5)/ALPHA
S(4)=-DLOG(.5)/BETA
S(5)=K10
S(6)=K12
S(7)=K21
S(8)=V
END
EOM
INIT 5.62,2.75,2.9873,0.1641
NCON 1
CONS 35
NOBS 8
DATA
BEGIN

PCNONLIN NONLINEAR ESTIMATION PROGRAM

ITERATION WEIGHTED SS     A          B          ALPHA      BETA

    0      .412316E-01    5.620      2.750      2.987      .1641

    1      .412088E-01    5.624      2.751      2.987      .1641

CONVERGENCE ACHIEVED

RELATIVE CHANGE IN WEIGHTED SUM OF SQUARES LESS THAN    .000100

    1      .412087E-01    5.625      2.751      2.988      .1641
```

Figure 33 (cont.). The output of example 5-fitting data to Model 8.

PCNONLIN NONLINEAR ESTIMATION PROGRAM

PARAMETER	ESTIMATE	STANDARD ERROR	95% CONFIDENCE LIMITS		
A	5.624518	.311032	4.760967	6.488069	UNIVARIATE
			4.029071	7.219965	PLANAR
B	2.751436	.181382	2.247845	3.255027	UNIVARIATE
			1.821030	3.681842	PLANAR
ALPHA	2.987688	.361232	1.984761	3.990615	UNIVARIATE
			1.134737	4.840639	PLANAR
BETA	.164119	.015873	.120049	.208188	UNIVARIATE
			.082698	.245539	PLANAR

PCNONLIN NONLINEAR ESTIMATION PROGRAM

*** CORRELATION MATRIX OF THE ESTIMATES ***

1.00000			
-.02045	1.00000		
.52781	.77671	1.00000	
-.07900	.84574	.59525	1.00000

*** EIGENVALUES OF (A TRANSPOSE A) MATRIX ***

NUMBER	EIGENVALUE
1	151.1
2	3.214
3	.1454
4	.5531E-01

PCNONLIN NONLINEAR ESTIMATION PROGRAM

*** SUMMARY OF NONLINEAR ESTIMATION ***

FUNCTION 1

X	OBSERVED Y	CALCULATED Y	RESIDUAL	WEIGHT	SD-YHAT	STANDARIZED RESIDUAL
.1670	6.103	6.092	.1080E-01	1.000	.1011	.1064
.5000	3.748	3.797	-.4932E-01	1.000	.9329E-01	-.4859
1.000	2.710	2.618	.9112E-01	1.000	.6694E-01	.8977
2.000	1.967	1.996	-.2935E-01	1.000	.7685E-01	-.2892
4.000	1.355	1.427	-.7234E-01	1.000	.5138E-01	-.7127
6.000	1.098	1.028	.7021E-01	1.000	.5431E-01	.6917
8.000	.6488	.7402	-.9141E-01	1.000	.5875E-01	-.9006
12.00	.4887	.3839	.1048	1.000	.5339E-01	1.032

Figure 33 (cont.). The output of example 5-fitting data to Model 8.

```
CORRECTED SUM OF SQUARED OBSERVATIONS =  25.1735
WEIGHTED CORRECTED SUM OF SQUARED OBSERVATIONS =  25.1735
SUM OF SQUARED RESIDUALS =            .412087E-01
SUM OF WEIGHTED SQUARED RESIDUALS =  .412087E-01
S =  .101500     WITH     4 DEGREES OF FREEDOM
CORRELATION (Y,YHAT) = .999


PCNONLIN NONLINEAR ESTIMATION PROGRAM

SUMMARY OF ESTIMATED SECONDARY PARAMETERS

PARAMETER          ESTIMATE        STANDARD
                                     ERROR

AUC              18.647493          .961647
K10-HL            1.543162          .110720
ALPHA-HL           .232001          .028023
BETA-HL           4.223453          .408067
K10                .449173          .032267
K12               1.610994          .215591
K21               1.091639          .150598
VOLUME            4.178628          .177916

PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION  1
PLOT OF X VS. OBSERVED Y AND CALCULATED Y

*** ARE CALCULATED POINTS, OOO ARE OBSERVED POINTS


  7.000       I
  6.500       I                  O
  6.000       I
  5.500       I                  *
  5.000       I
  4.500       I                  *
  4.000       I                  O*
  3.500       I                   *
  3.000       I                   O
  2.500       I                    **
  2.000       I                     O****
  1.500       I                         O***O*
  1.000       I                              ***O*****
  .5000       I                                    ***O
  .0000       I
 -.5000       I
              -----+---------+---------+---------+---------+---------+-----
                 -6.00      -1.00       4.00       9.00       14.0       19.0
                                          X
```

Figure 33 (cont.). The output of example 5-fitting data to Model 8.

```
PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION  1
PLOT OF OBSERVED Y VS. CALCULATED Y

CALCULATED Y
  7.000       I
  6.500       I                                                    *
  6.000       I
  5.500       I
  5.000       I
  4.500       I
  4.000       I                                        *
  3.500       I
  3.000       I                                  *
  2.500       I
  2.000       I                            *
  1.500       I                         **
  1.000       I                       *
  .5000       I                     *
  .0000       I
 -.5000       I
              -----+---------+---------+---------+---------+---------+-----
                 -1.60       .400       2.40       4.40       6.40       8.40
                                      OBSERVED Y

PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION  1
PLOT OF CALCULATED Y VS. RESIDUAL

RESIDUAL
  .1600       I
  .1400       I
  .1200       I                   *
  .1000       I                              *
  .8000E-01   I                         *
  .6000E-01   I
  .4000E-01   I
  .2000E-01   I                                                    *
  .0000       I     ---------------------------------------------------------
 -.2000E-01   I                              *
 -.4000E-01   I                                        *
 -.6000E-01   I                         *
 -.8000E-01   I                    *
 -.1000       I
 -.1200       I
 -.1400       I
              -----+---------+---------+---------+---------+---------+-----
                 -1.60       .400       2.40       4.40       6.40       8.40
                                      CALCULATED Y
```

Figure 33 (cont.). The output of example 5-fitting data to Model 8.

```
PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION  1
PLOT OF X VS. RESIDUAL Y


RESIDUAL
  .4000        I
  .3500        I
  .3000        I
  .2500        I
  .2000        I
  .1500        I                                        *
  .1000        I                    *          *
  .5000E-01    I                   *
  .0000        I     ---------------*--*-----------------------------------
 -.5000E-01    I                         *        *
 -.1000        I
 -.1500        I
 -.2000        I
 -.2500        I
 -.3000        I
 -.3500        I
               -----+---------+---------+---------+---------+---------+-----
                  -6.00      -1.00       4.00       9.00       14.0       19.0
                                                 X
```

```
PCNONLIN NONLINEAR ESTIMATION PROGRAM V01-E

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 LISTING OF INPUT COMMANDS

 FINISH


 NORMAL ENDING
```

Figure 33 (cont.). The output of example 5-fitting data to Model 8.

APPENDIX I

Table 18. Clinical responses of patients: Diagnosis, tumor size and tumor response.

Patients no.	Diagnosis for cancer of	Tumor sizes (cm)		Node sizes (cm)		Tumor responses
		before treatment	after treatment	before treatment	after treatment	
1	nasopharynx	NM	NM	10 X 6	7 X 4	NM
2	lower gum	3.3	1.2	-	-	PR
3	base of tongue	1.1	0	-	-	CR
4	soft palate	2.2	0.9	-	-	PR
5	buccal mucosa	5.8	NF	-	-	NF
6	base of tongue	1.2	0	-	-	CR
7	tongue	1.9	0	-	-	CR
8	pharynx	3.0	1.3	6 X 7	5 X 5	PR
9	tongue	1.4	0	-	-	CR
10	metastatic cancer	NM	NM	7 X 6	5 X 5	NM
11	nasopharynx	NM	NM	-	-	NM

NM = tumor size could not be measured

NF = patient did not followed up

CR = complete, PR = partial response

VITAE

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