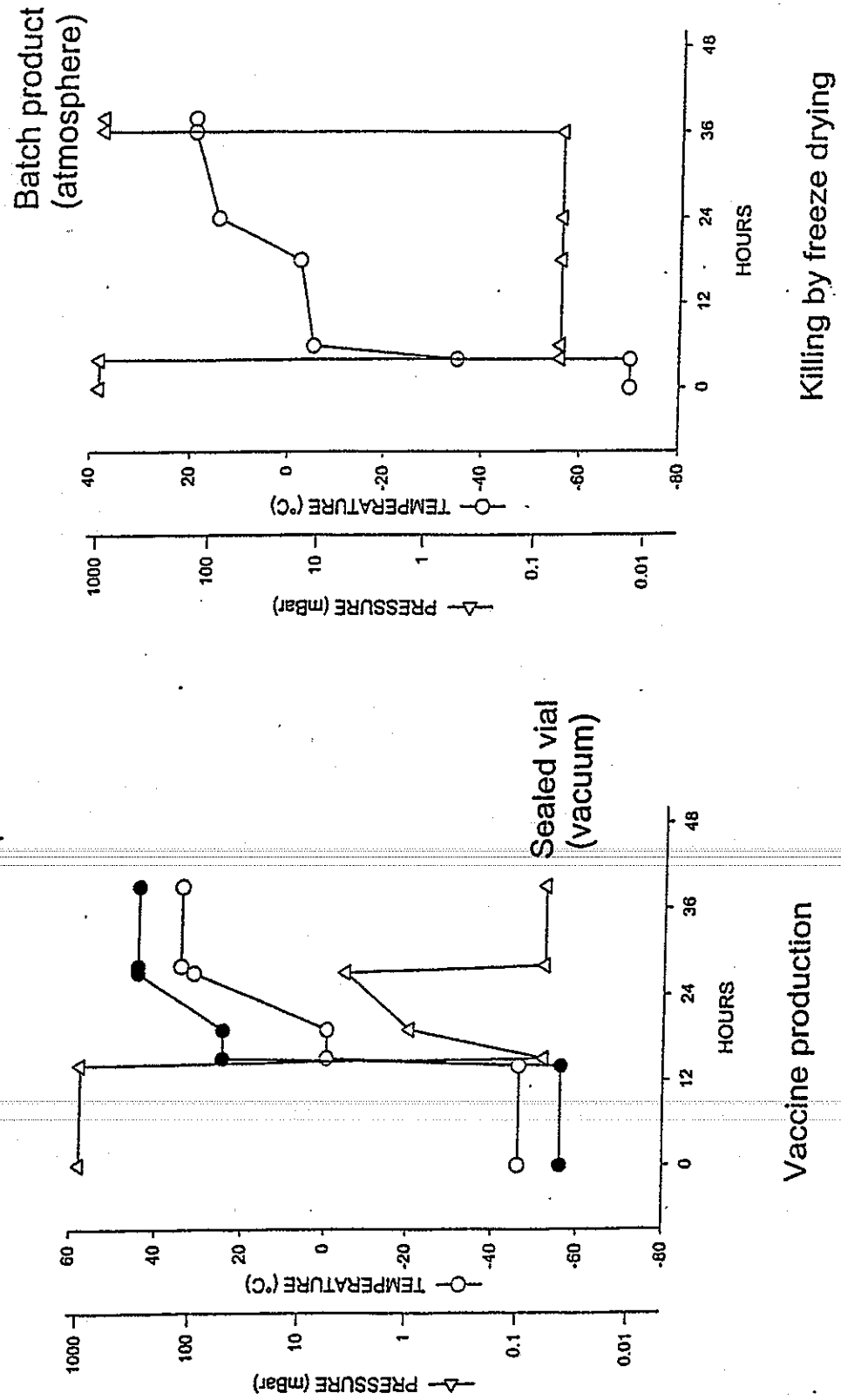

Batch product
(atmosphere)
PRESSURE (mBar)
TEMPERATURE (°C)
HOURS
Killing by freeze drying
Sealed vial
(vacuum)
PRESSURE (mBar)
TEMPERATURE (°C)
HOURS
Vaccine production

FIGURE 1

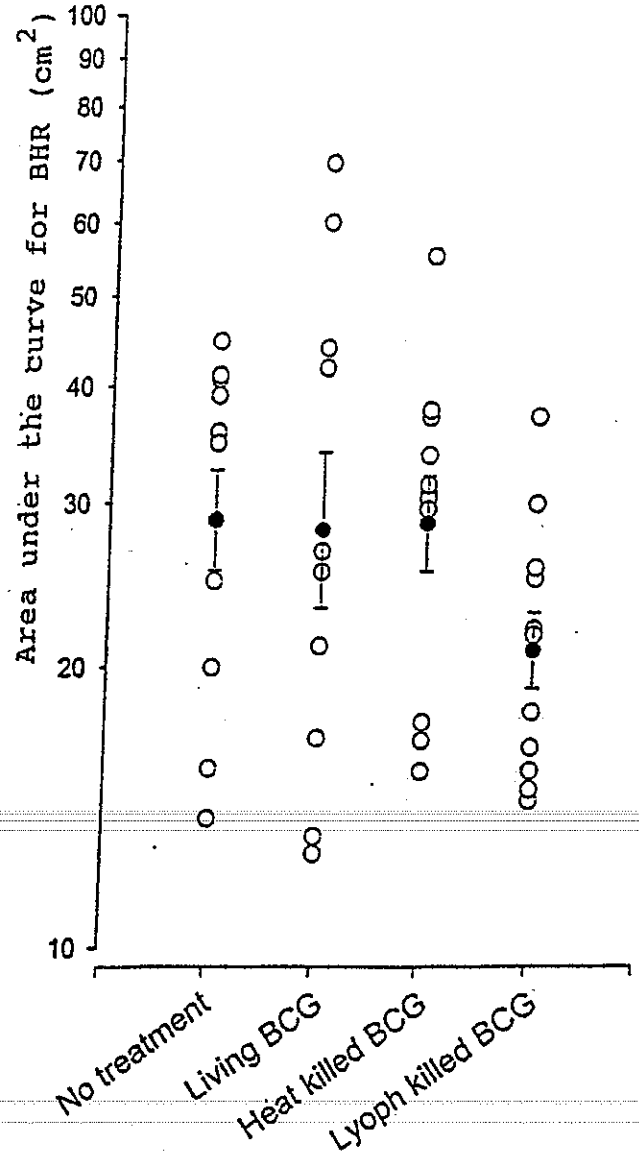

Area under the curve for BHR (cm2)
100
90
80
70
60
50
40
30
20
10
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 2

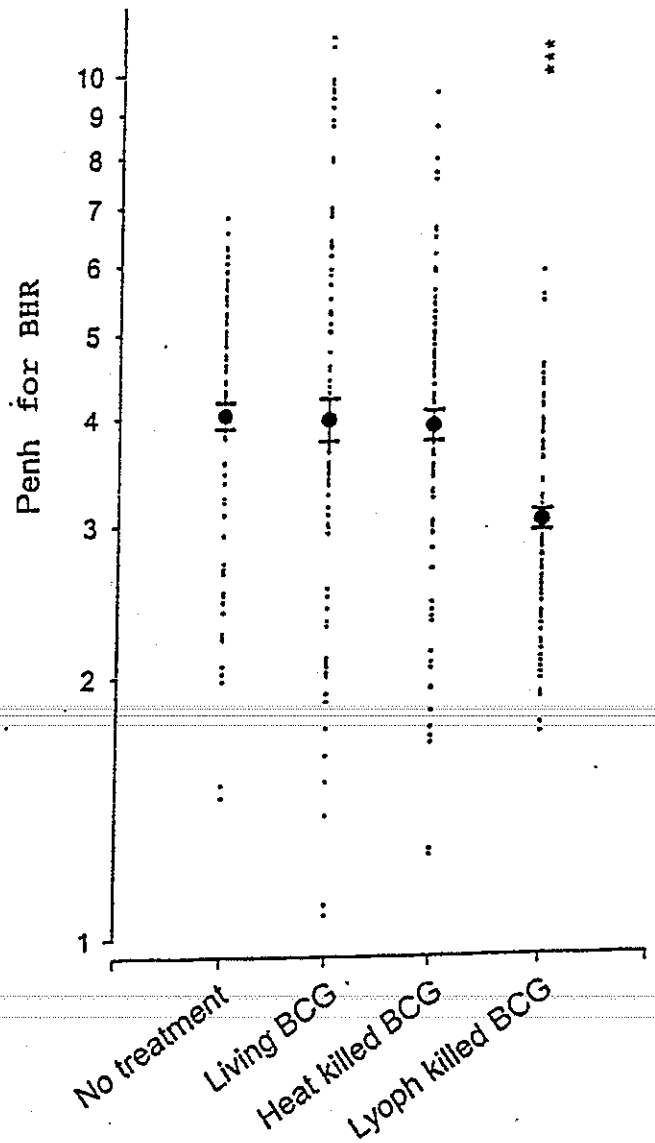

Penh for BHR
10
9
8
7
6
5
4
3
2
1
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 3

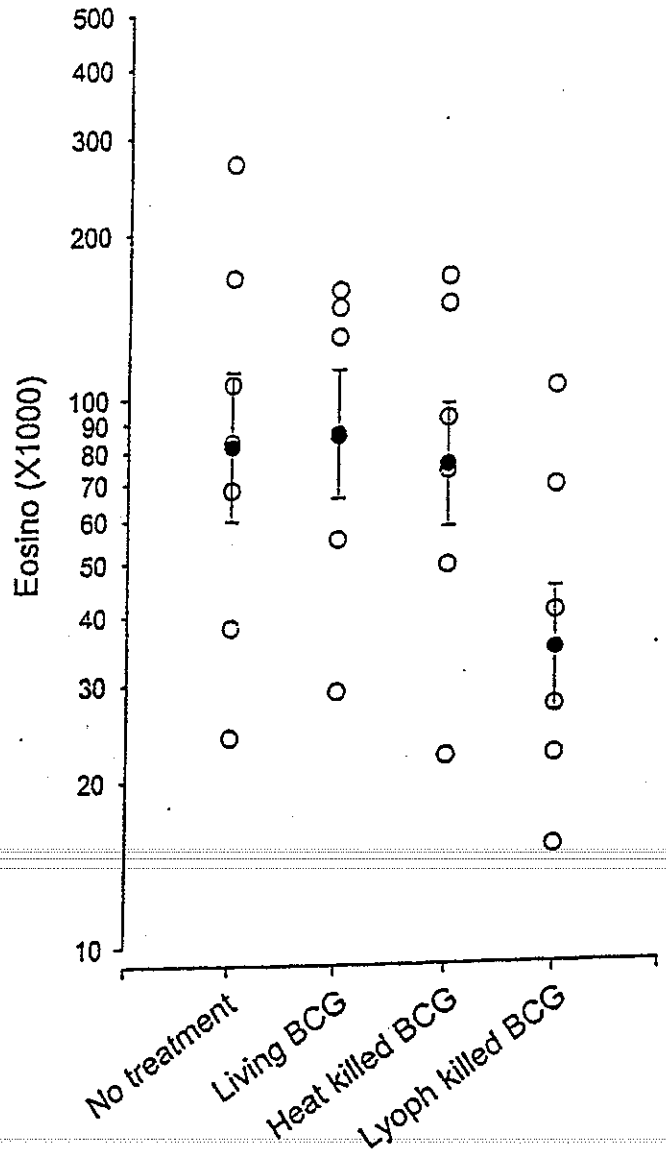

Eosino (X1000)
500
400
300
200
100
90
80
70
60
50
40
30
20
10
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 4

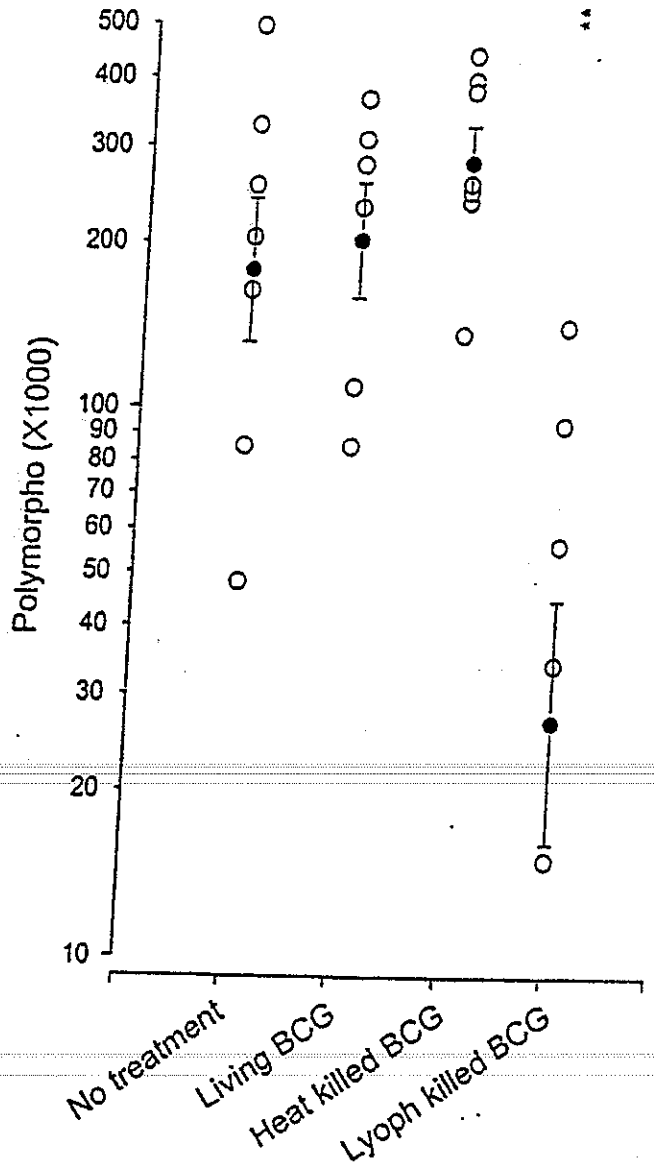

Polymorpho (X1000)
500
400
300
200
100
90
80
70
60
50
40
30
20
10
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 5

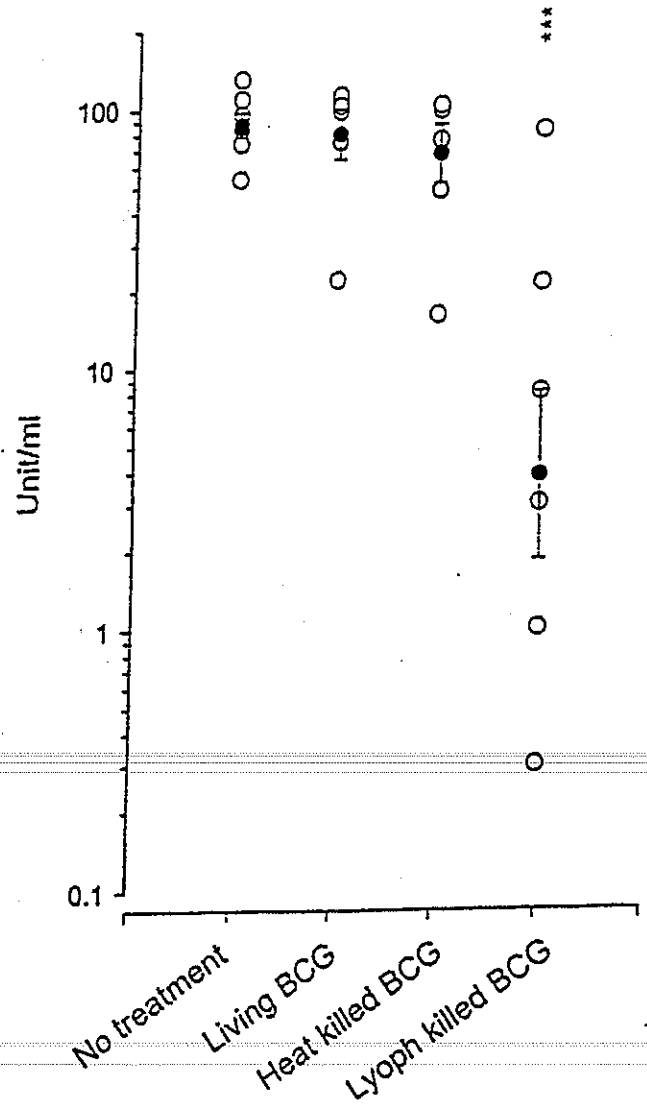

***
100
10
1
0.1
Unit/ml
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 6

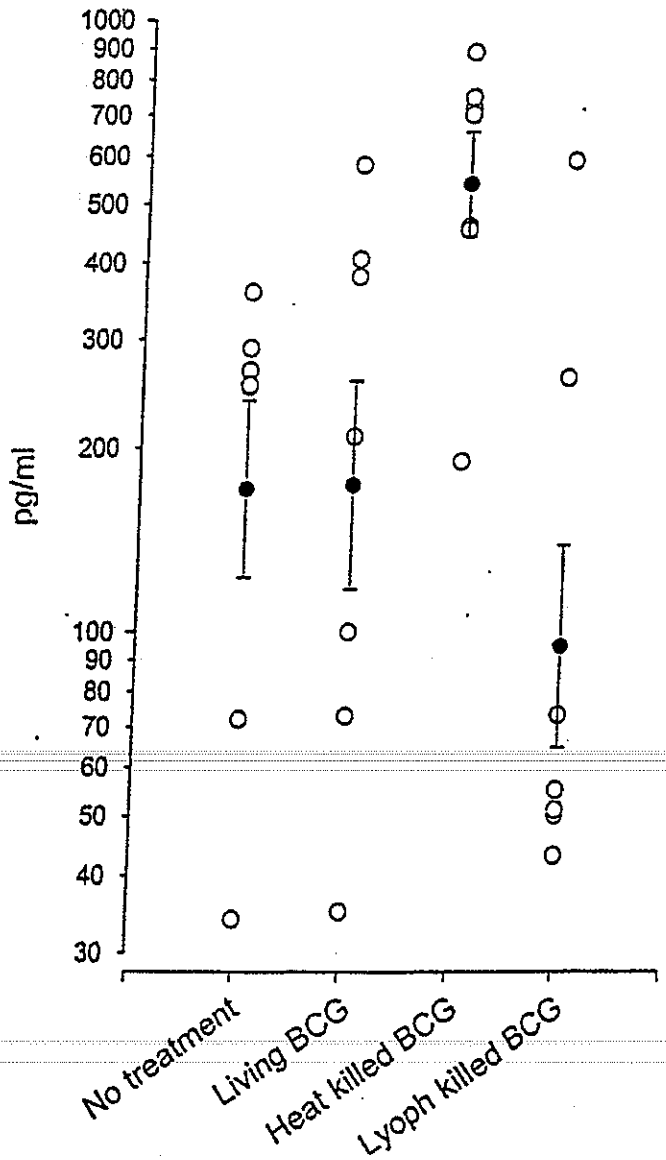

1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
pg/ml
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 7

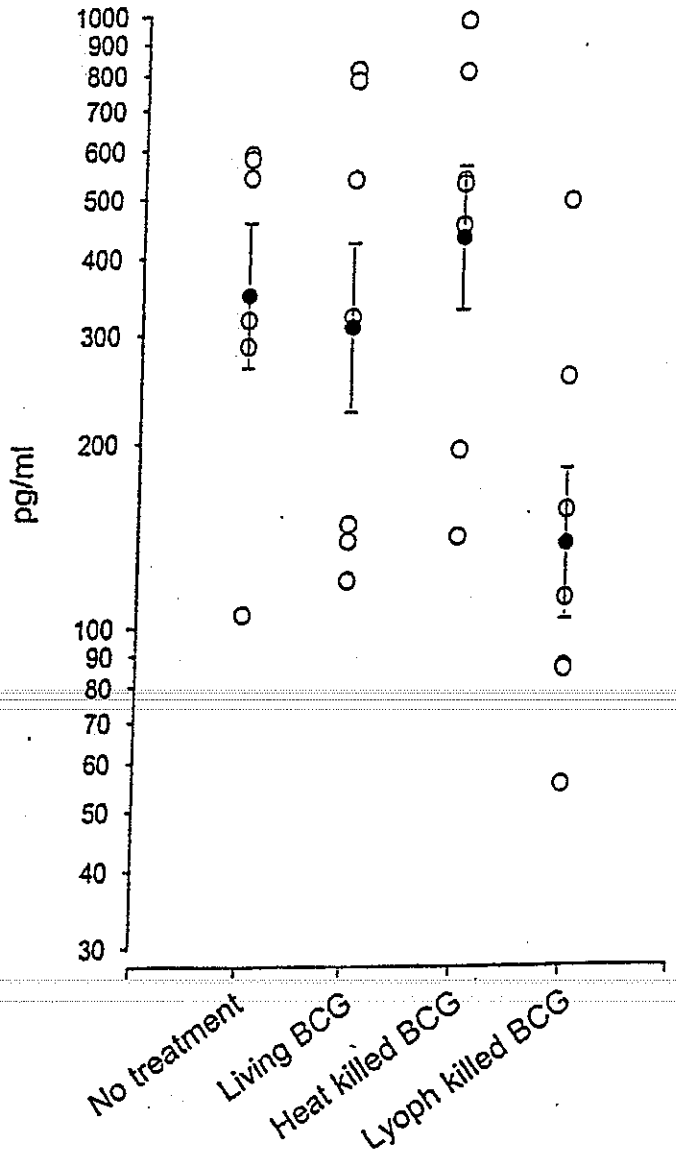

1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
pg/ml
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 8

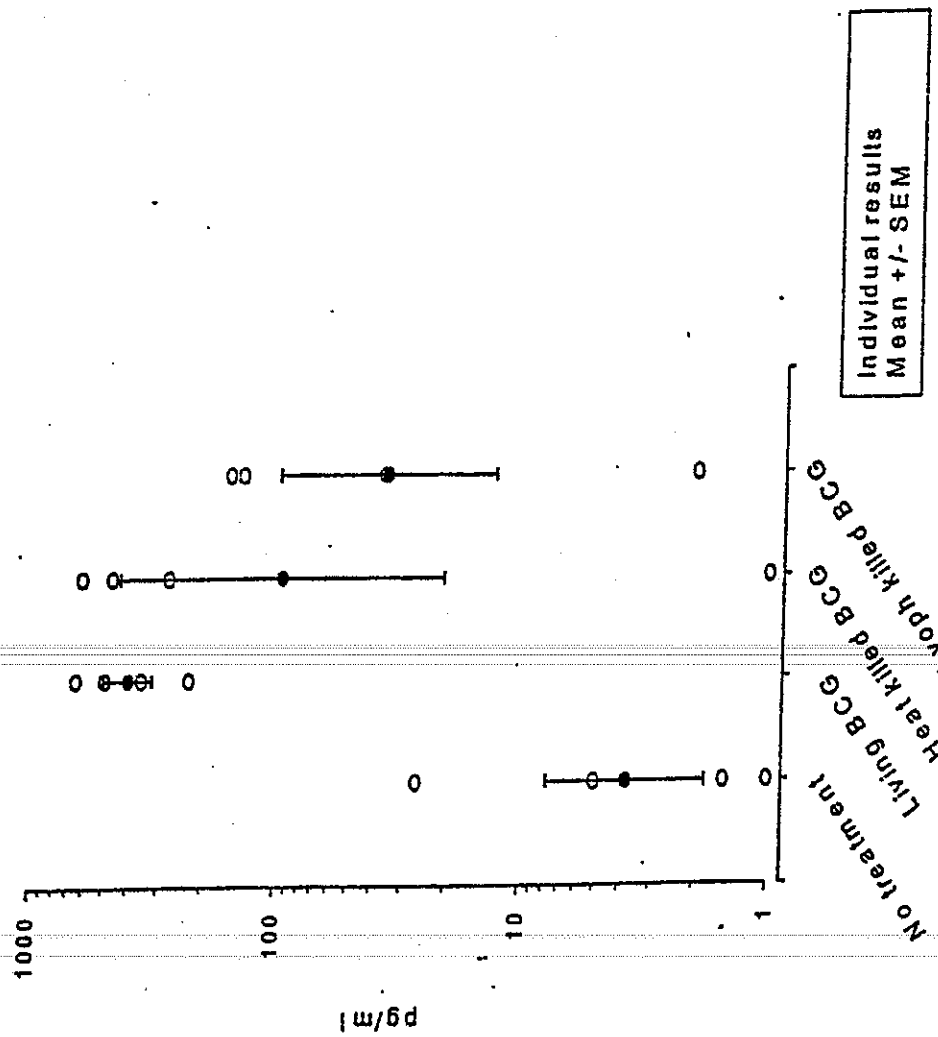

Individual results
Mean +/- SEM
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG
1000
100
10
1
pg/ml

FIGURE 9A

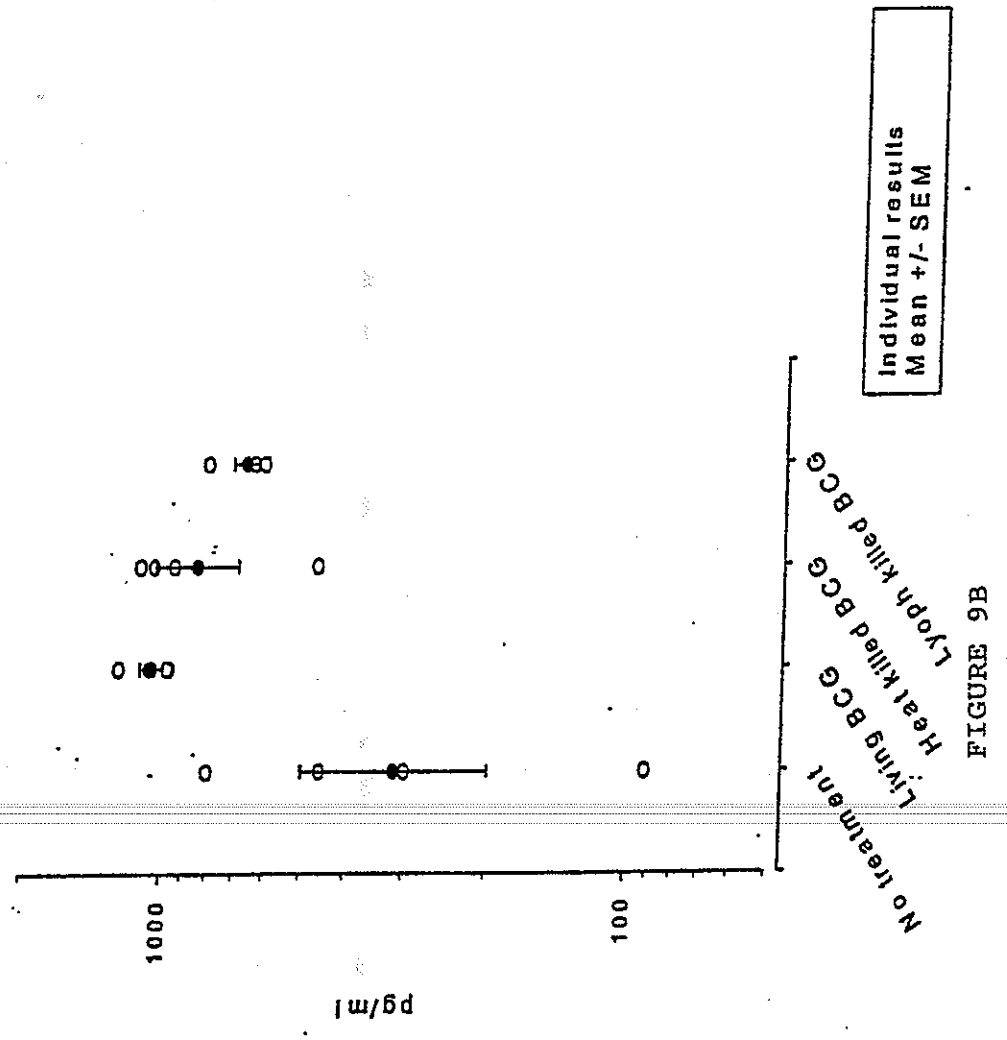

Individual results
Mean +/- SEM
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG
1000
100
pg/ml

FIGURE 9B

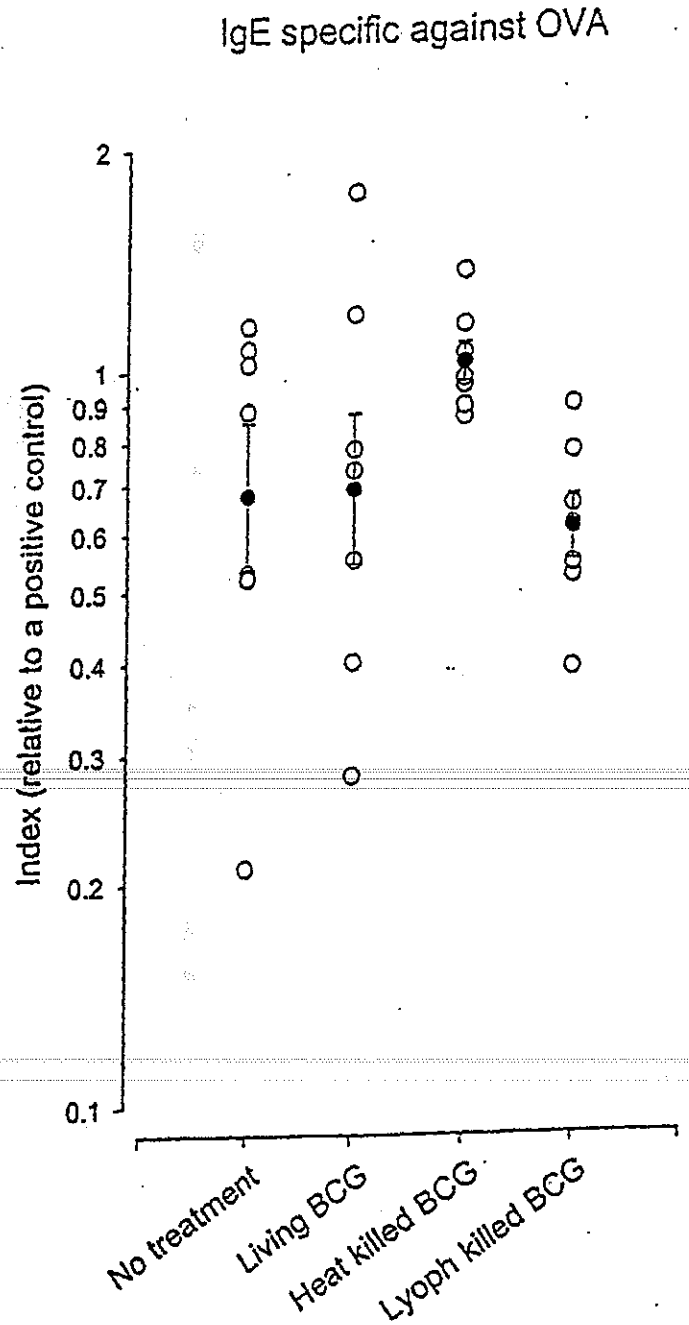

IgE specific against OVA
Index (relative to a positive control)
2
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 10

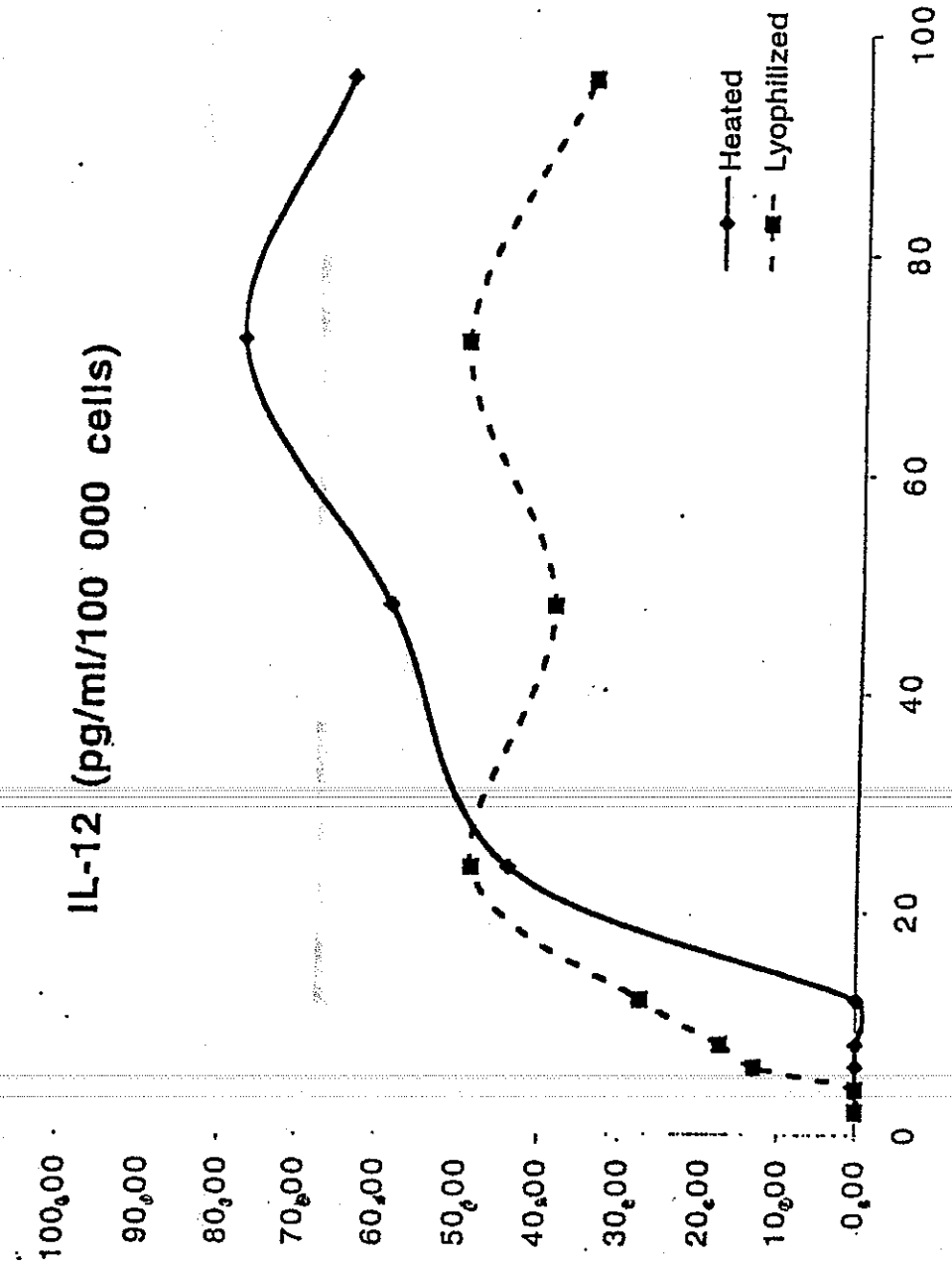

IL-12 (pg/ml/100 000 cells)
100,00
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
0
20
40
60
80
100
Heated
Lyophilized

FIGURE 11A

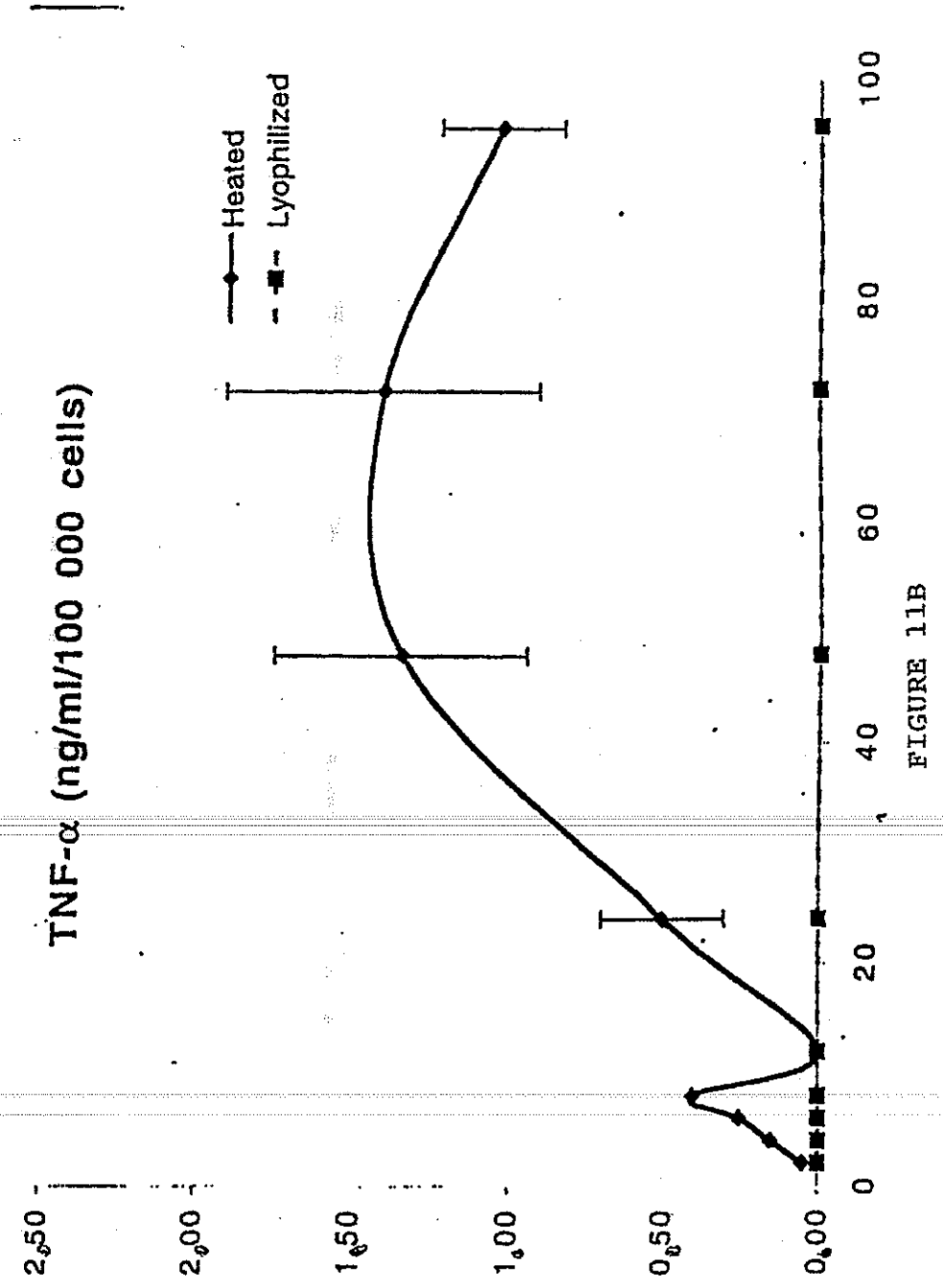

TNF-α (ng/ml/100 000 cells)
Heated
Lyophilized
2,50
2,00
1,50
1,00
0,50
0,00
0
20
40
60
80
100

FIGURE 11B

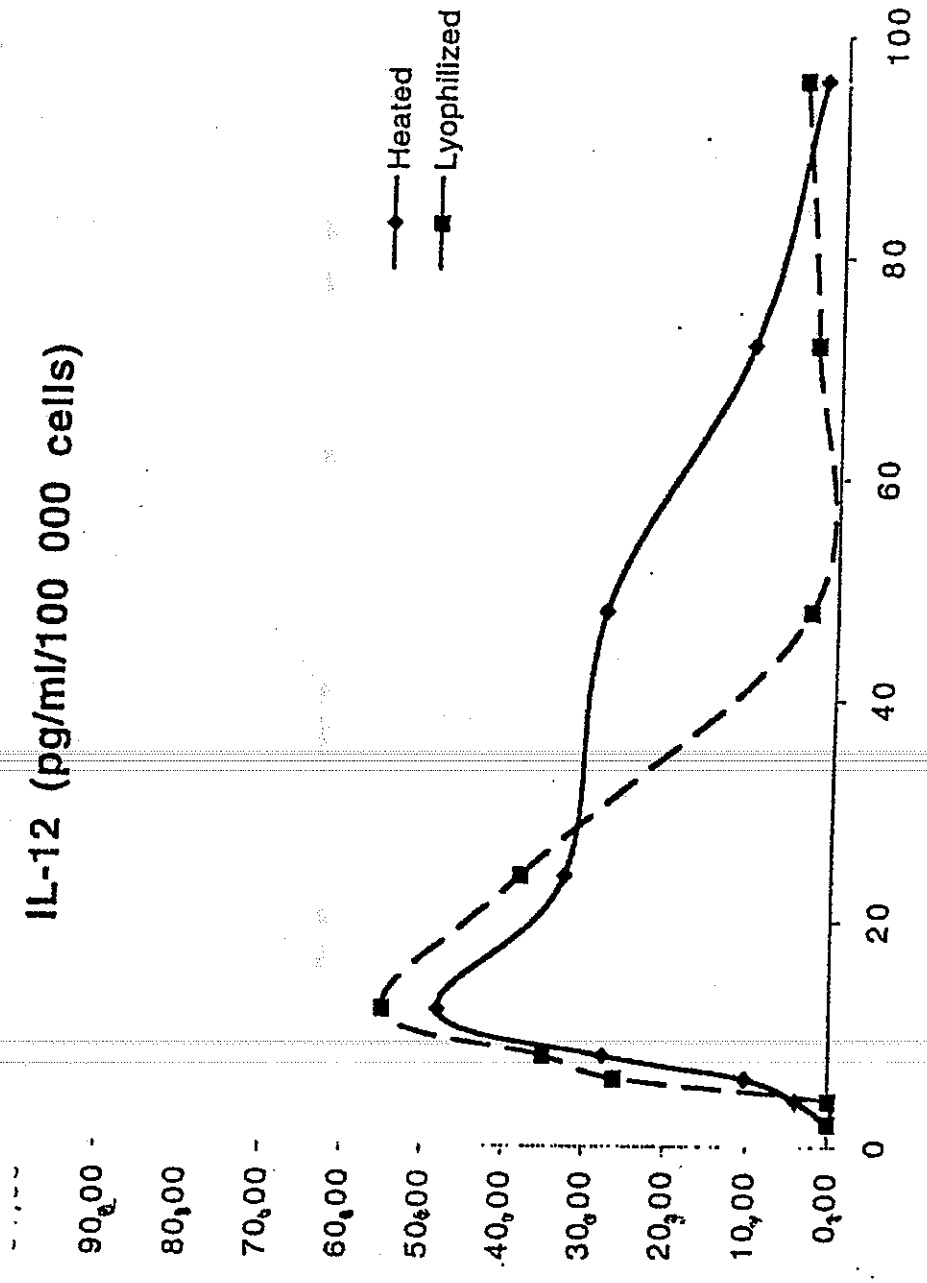

IL-12 (pg/ml/100 000 cells)
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
0
20
40
60
80
100
Heated
Lyophilized

FIGURE 12A

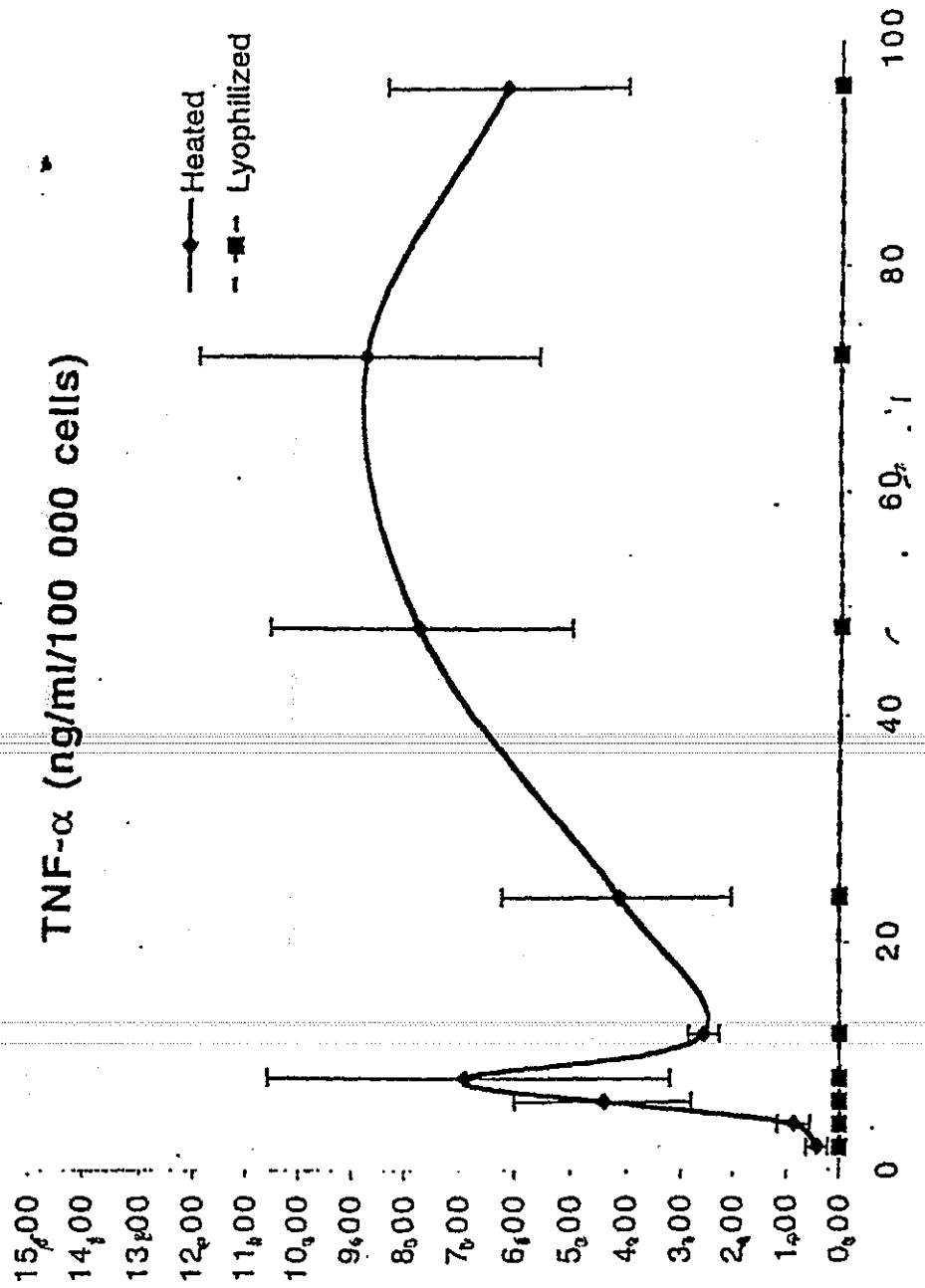

TNF-α (ng/ml/100 000 cells)
Heated
Lyophilized
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00
9,00
10,00
11,00
12,00
13,00
14,00
15,00
0
20
40
60
80
100

FIGURE 12B

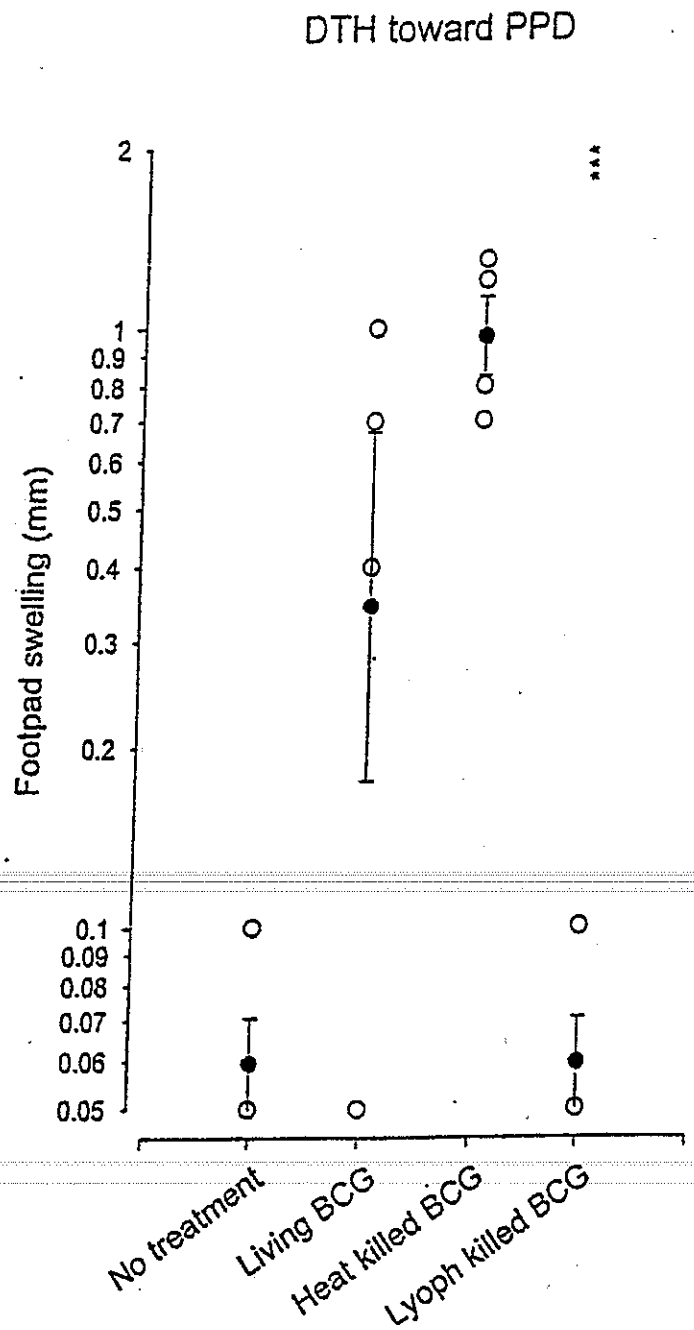

DTH toward PPD
Footpad swelling (mm)
2
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.09
0.08
0.07
0.06
0.05
***
No treatment
Living BCG
Heat killed BCG
Lyoph killed BCG

FIGURE 13

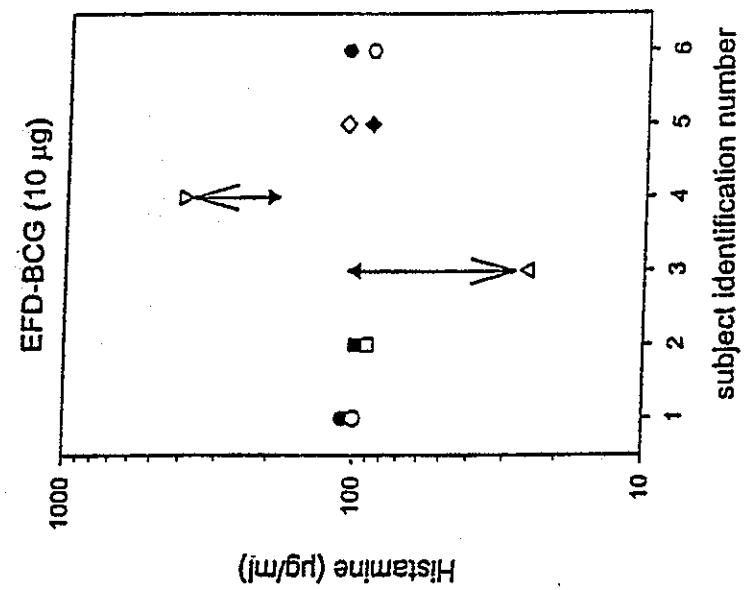


FIGURE 14B

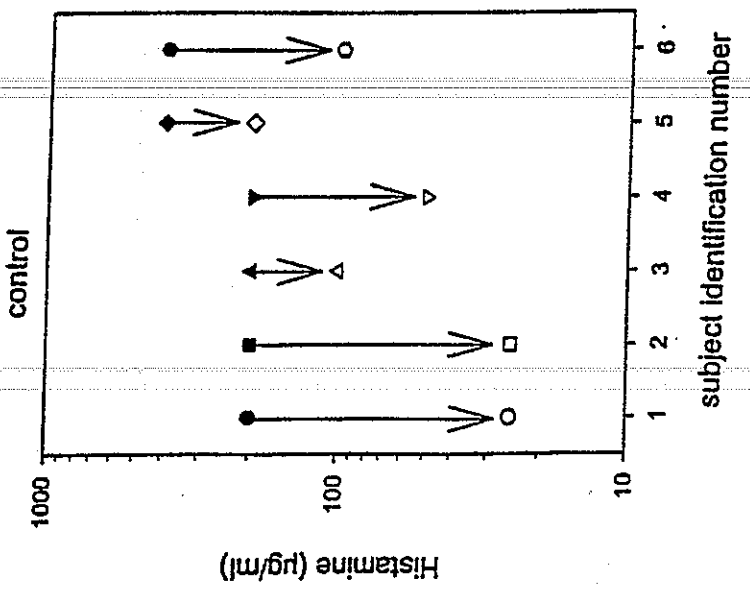


FIGURE 14A

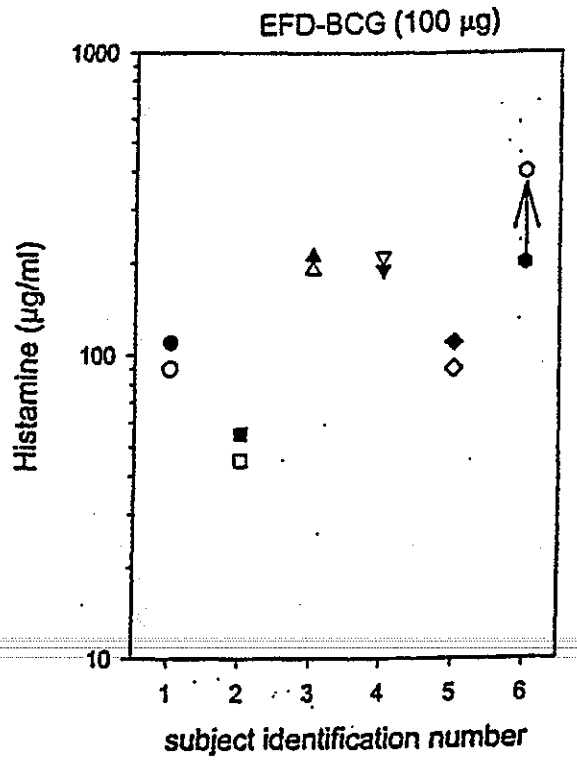

EFD-BCG (100 µg)
1000
100
10
Histamine (µg/ml)
1
2
3
4
5
6
subject identification number

FIGURE 14C

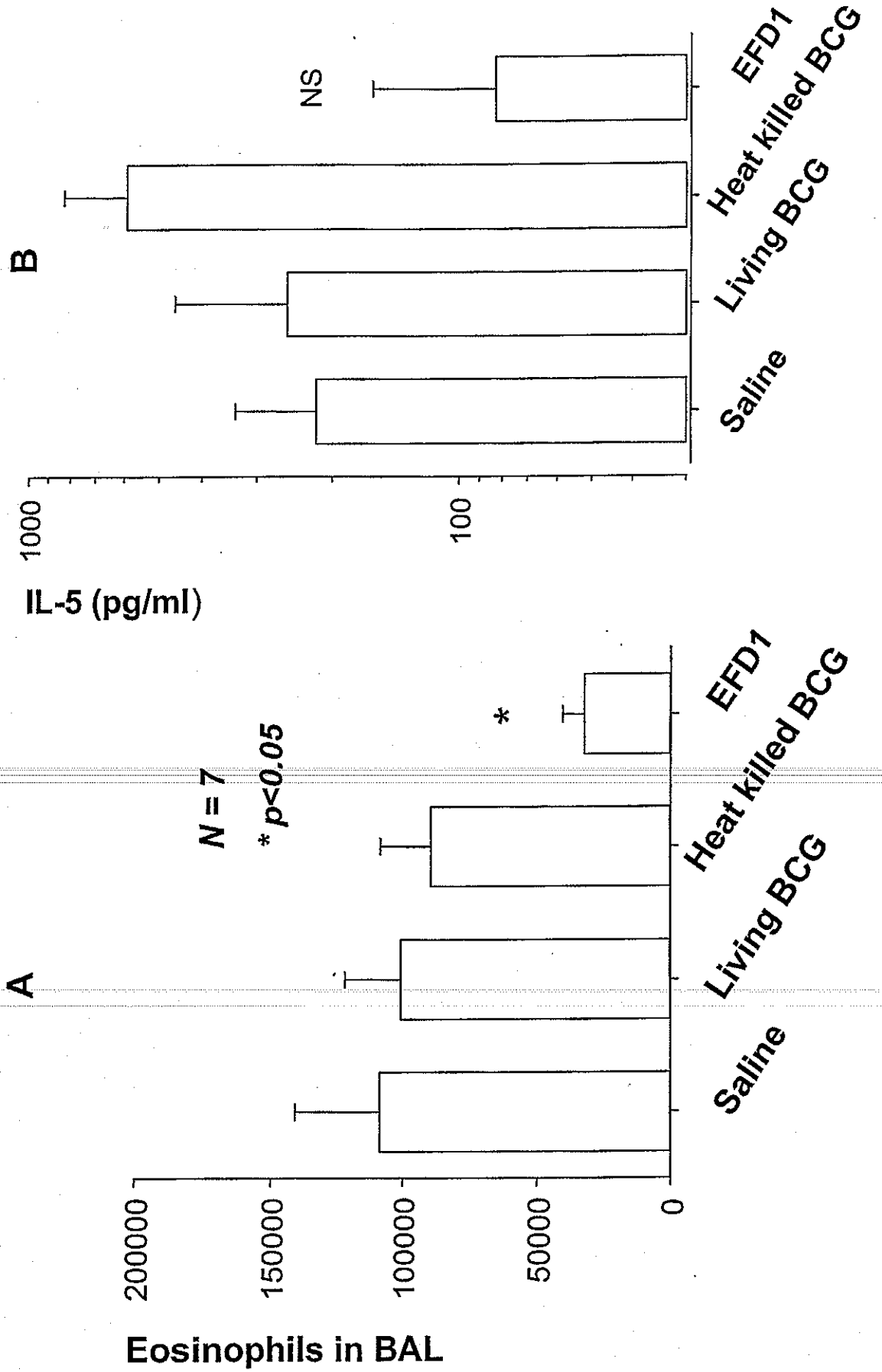

B
NS
EFD1
Heat killed BCG
Living BCG
Saline
1000
100
IL-5 (pg/ml)
A
N = 7
* p<0.05
*
EFD1
Heat killed BCG
Living BCG
Saline
200000
150000
100000
50000
0
Eosinophils in BAL

Figure 15

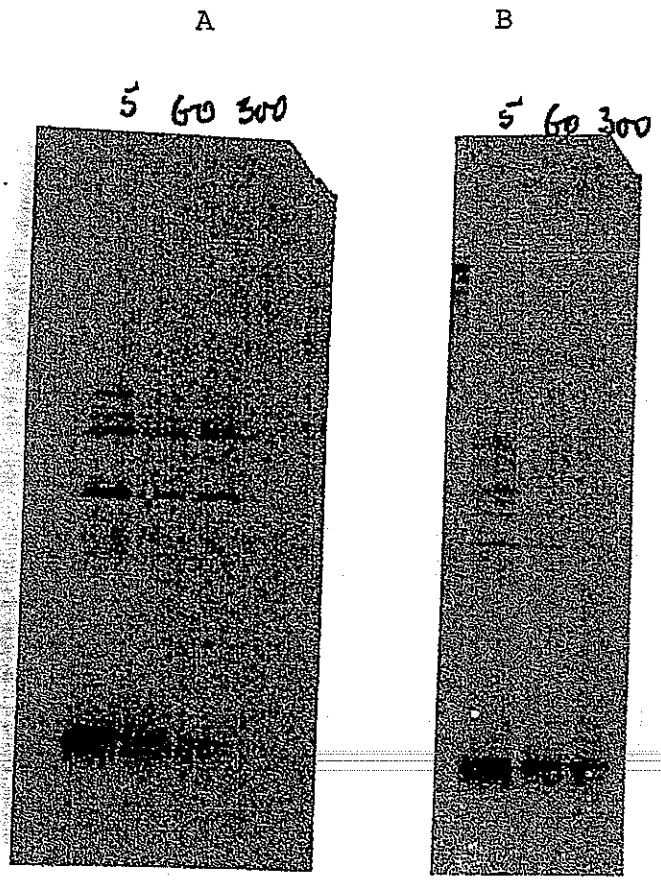


Figure 16

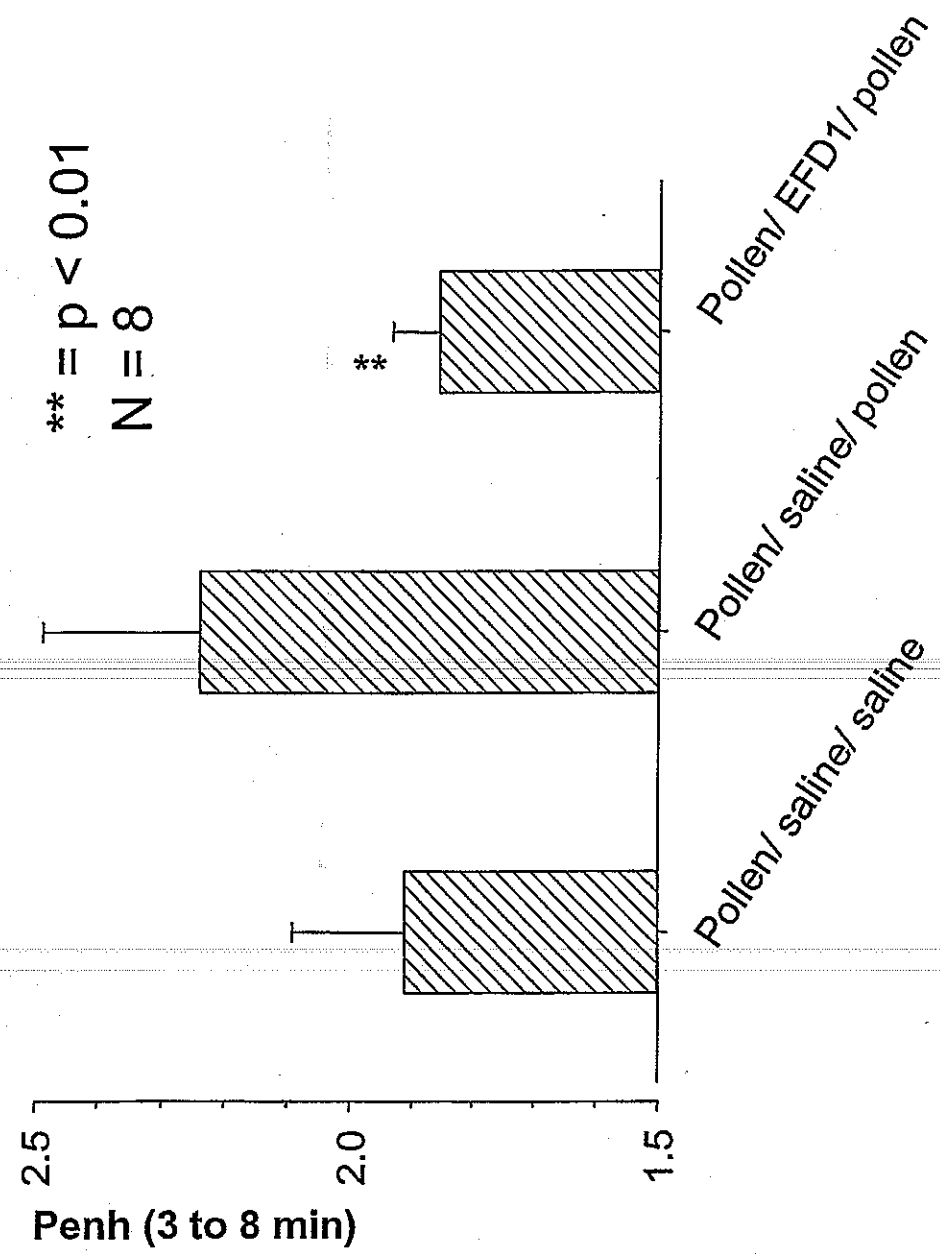

** = p < 0.01
N = 8
**
Pollen/ EFD1/ pollen
Pollen/ saline/ pollen
Pollen/ saline/ saline
2.5
2.0
1.5
Penh (3 to 8 min)

Figure 17

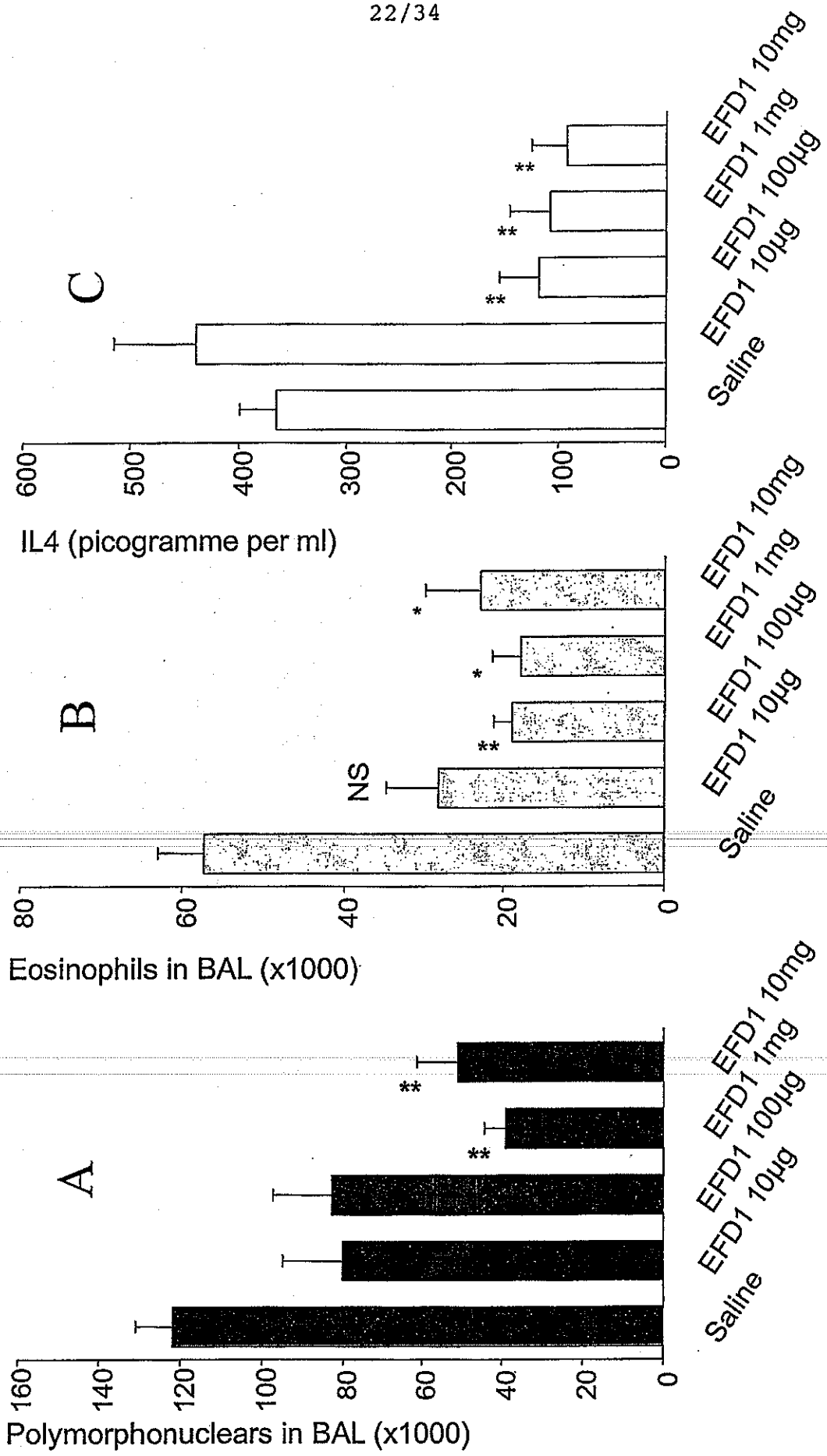

A
Polymorphonuclears in BAL (x1000)
160
140
120
100
80
60
40
20
0
**
**
EFD1 10mg
EFD1 1mg
EFD1 100µg
EFD1 10µg
Saline
B
Eosinophils in BAL (x1000)
80
60
40
20
0
*
*
**
NS
EFD1 10mg
EFD1 1mg
EFD1 100µg
EFD1 10µg
Saline
C
IL4 (picogramme per ml)
600
500
400
300
200
100
0
**
**
**
EFD1 10mg
EFD1 1mg
EFD1 100µg
EFD1 10µg
Saline

Figure 18

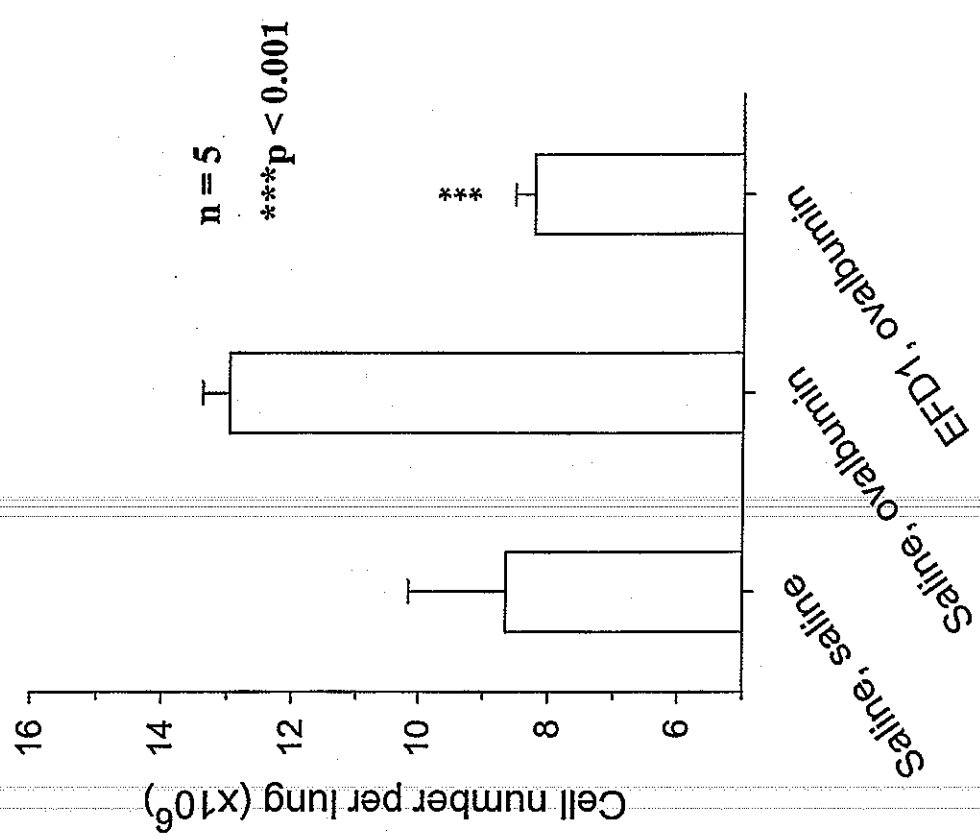
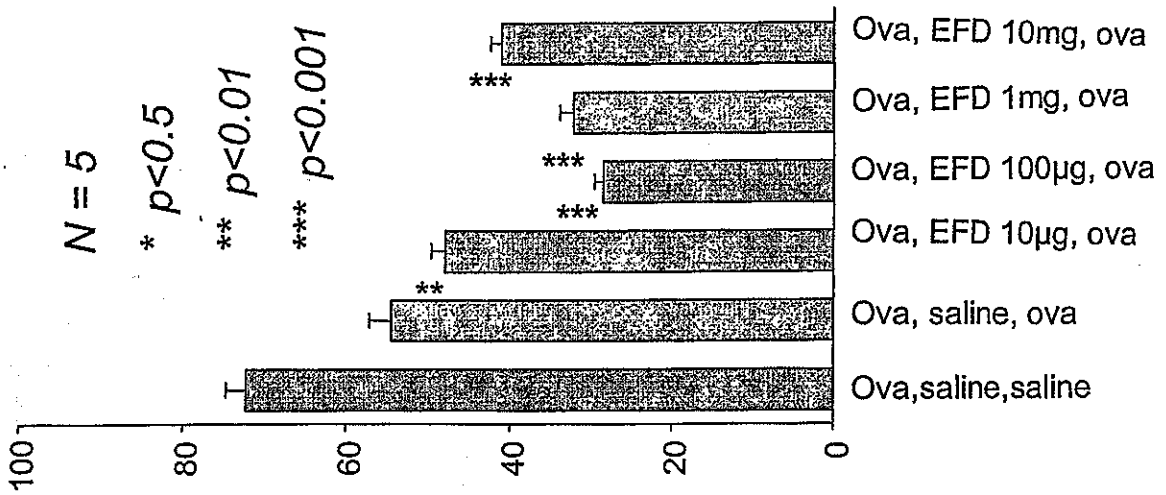
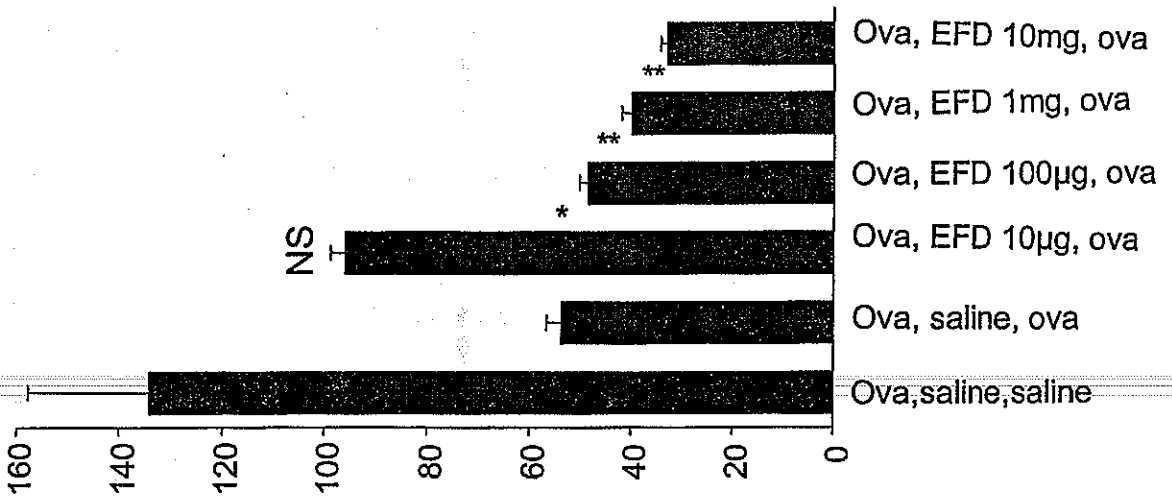

n = 5
***p < 0.001
***
Cell number per lung (x10^6)
16
14
12
10
8
6
Saline, saline
Saline, ovalbumin
EFD1, ovalbumin

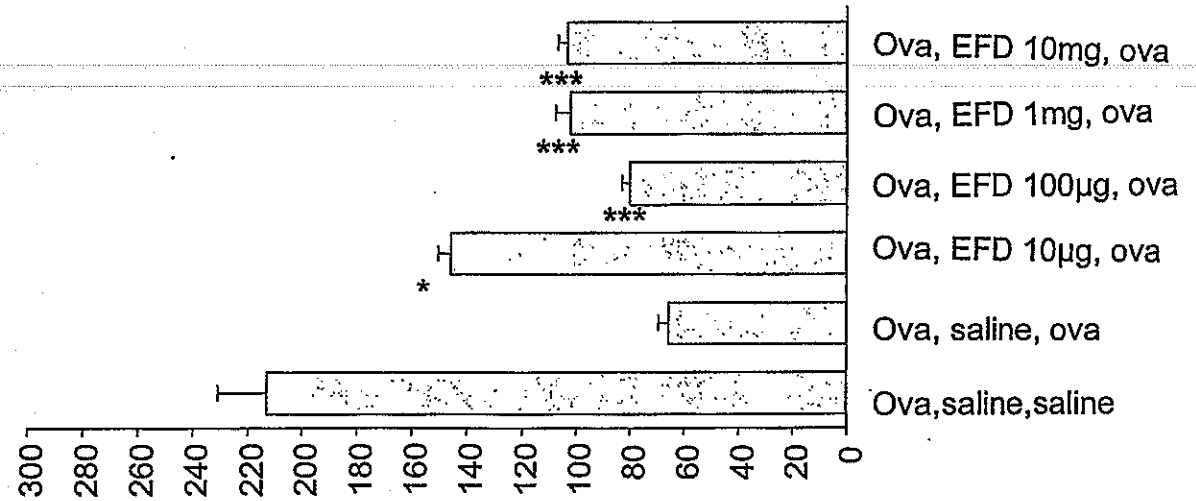
Figure 19



Dendritic cells (CD11c) (x1000)



Polymorphonuclear (Gr1+) (x1000)



Macrophages (CD11b) (x1000)

Figure 20

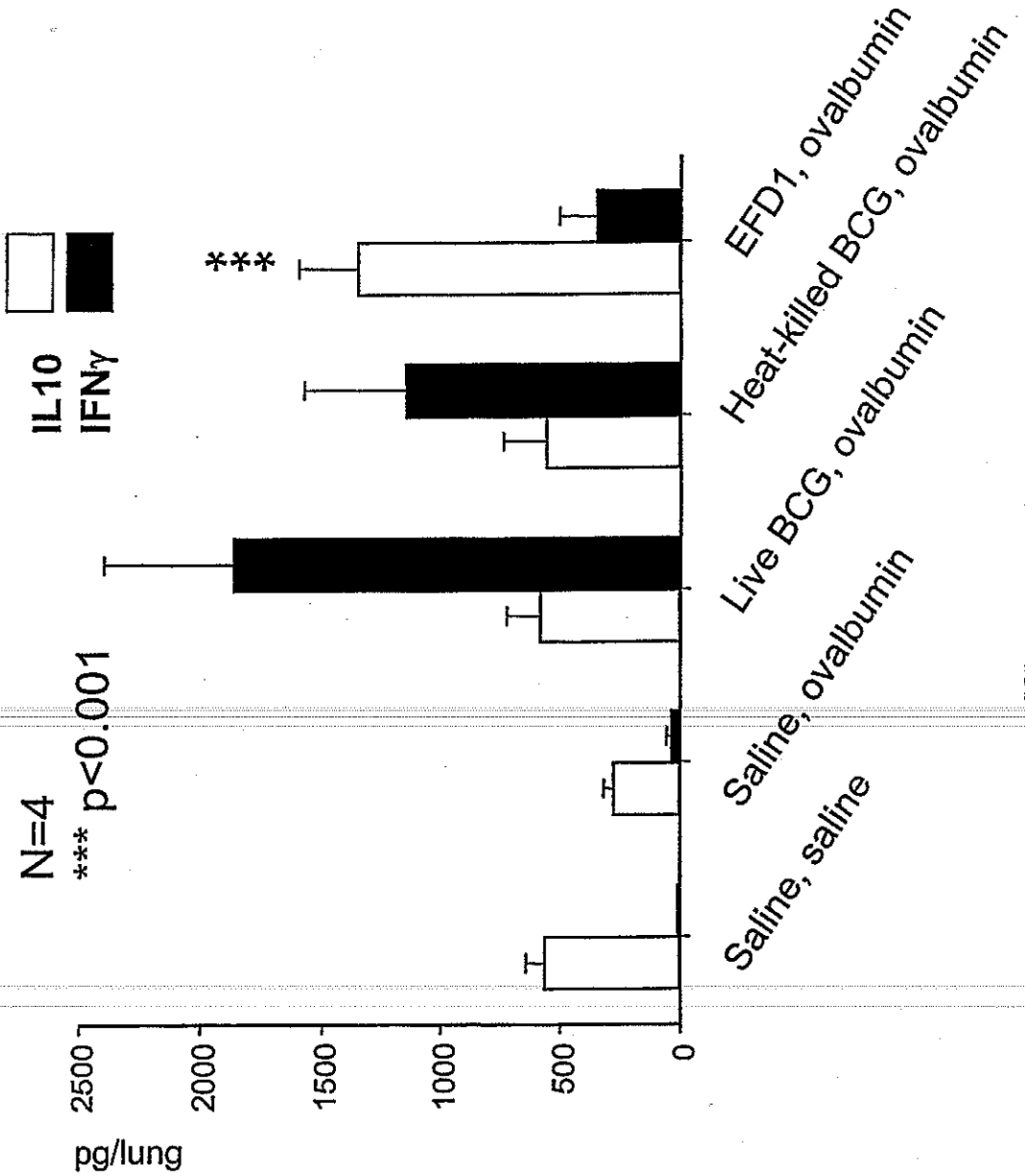

IL10
IFNγ
***
N=4
*** p<0.001
2500
2000
1500
1000
500
0
pg/lung
EFD1, ovalbumin
Heat-killed BCG, ovalbumin
Live BCG, ovalbumin
Saline, ovalbumin
Saline, saline

Figure 21

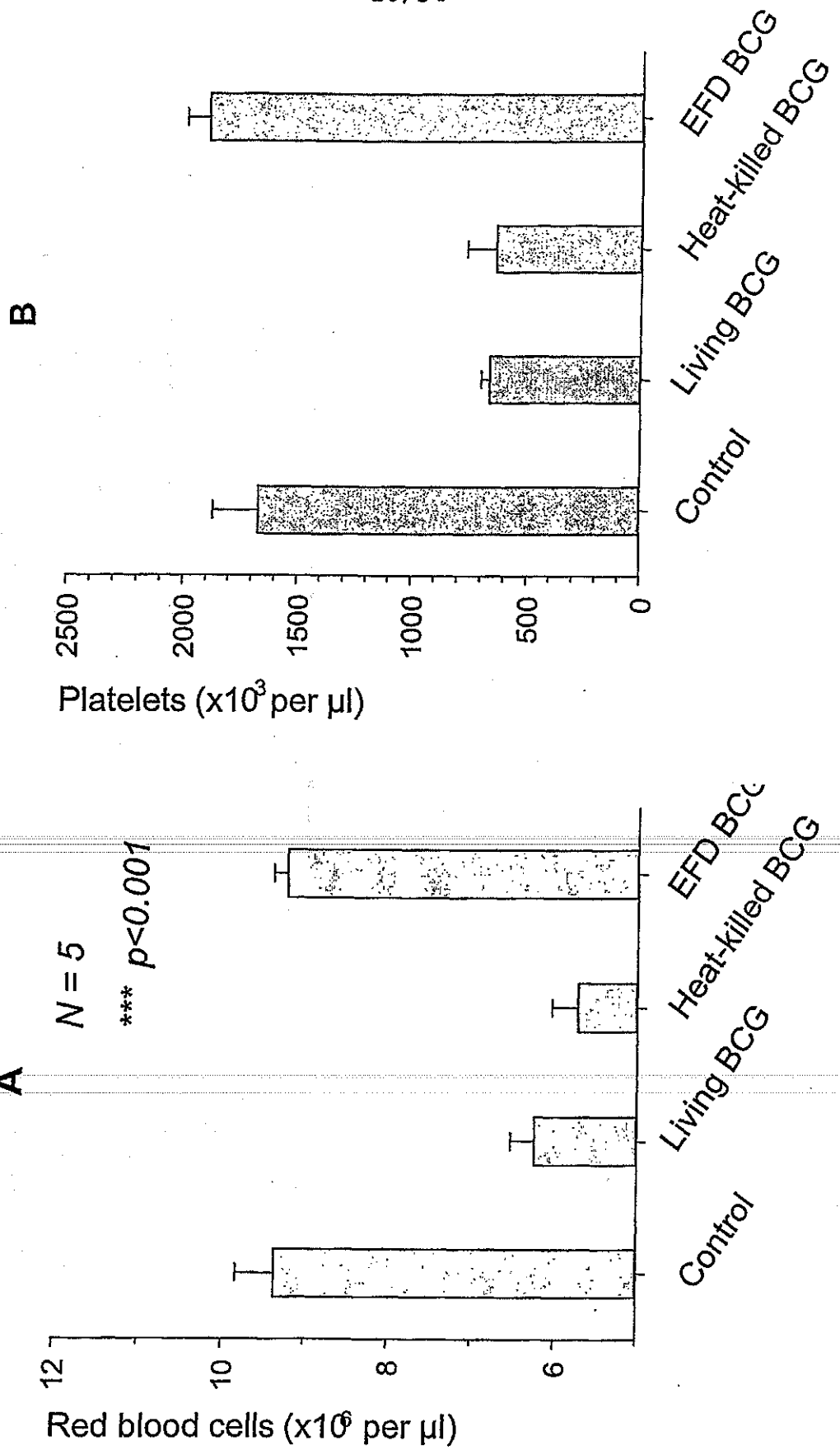

B
EFD BCG
Heat-killed BCG
Living BCG
Control
2500
2000
1500
1000
500
0
Platelets (x10³ per µl)
A
N = 5
*** p<0.001
EFD BCG
Heat-killed BCG
Living BCG
Control
12
10
8
6
Red blood cells (x10⁶ per µl)

Figure 22

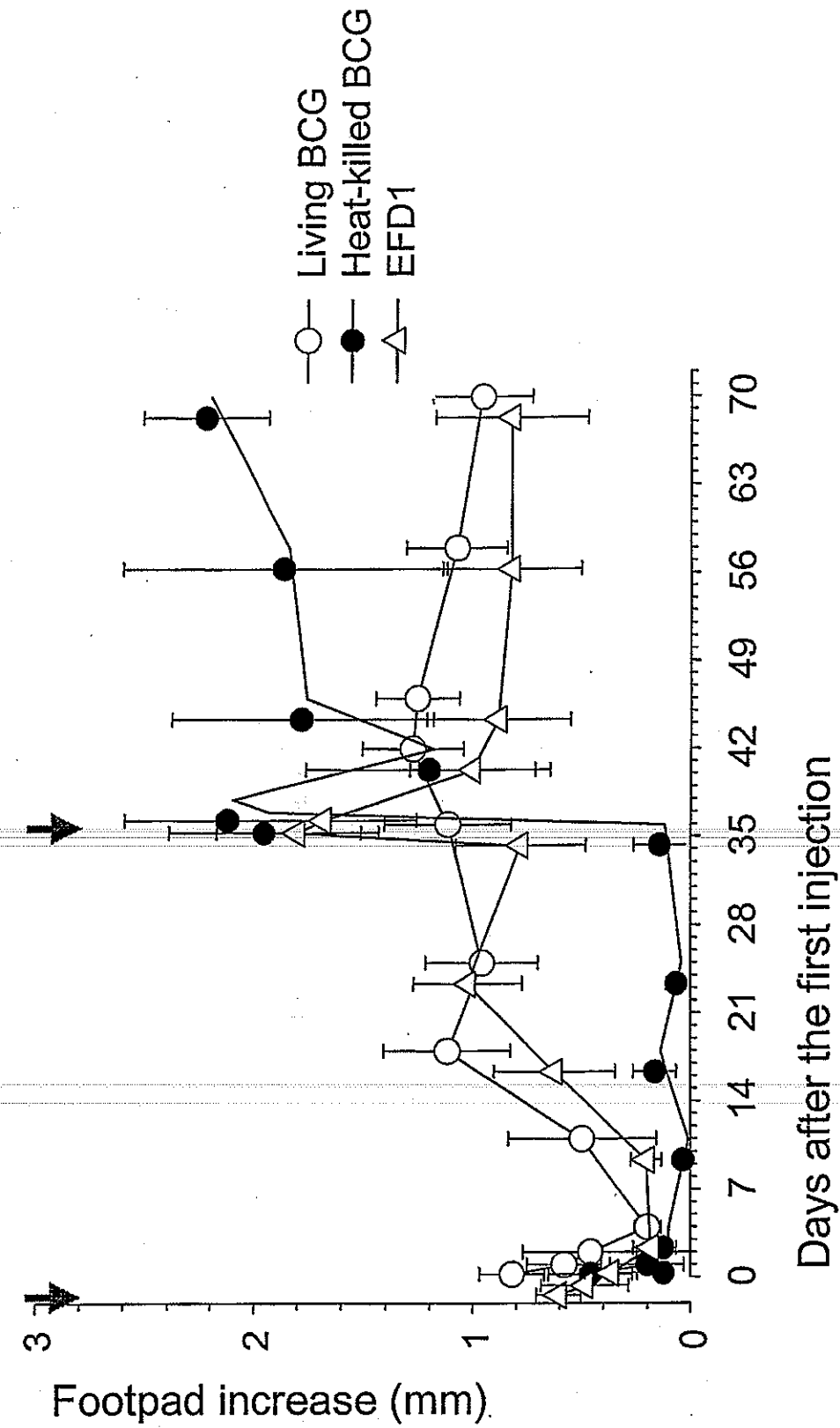

Living BCG
Heat-killed BCG
EFD1
Footpad increase (mm)
0
1
2
3
0
7
14
21
28
35
42
49
56
63
70
Days after the first injection

Figure 23

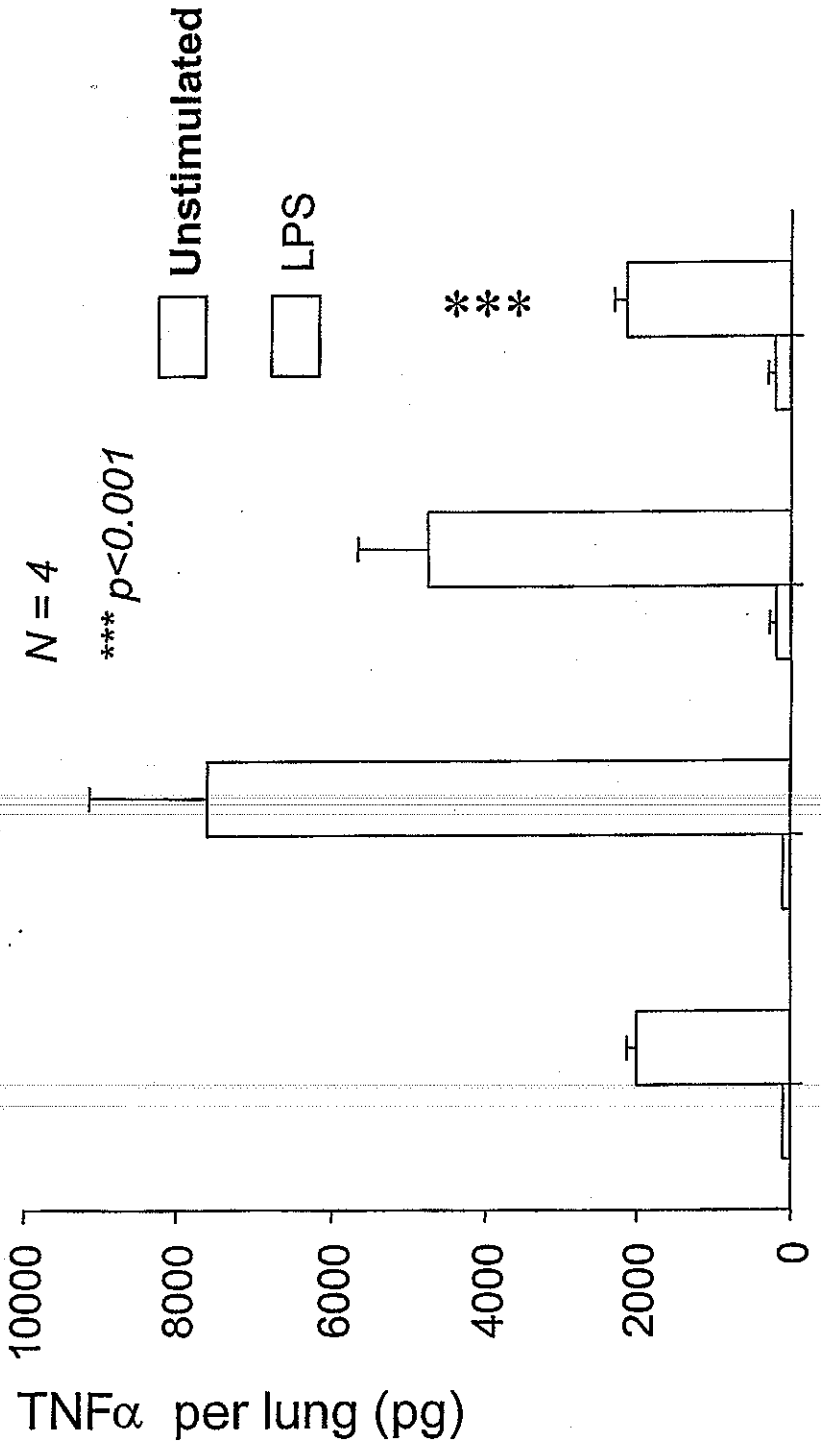

Unstimulated
LPS
***
N = 4
*** p<0.001
EFD1
Heat-killed BCG
Living BCG
Saline
10000
8000
6000
4000
2000
0
TNFα per lung (pg)

Figure 24


CD11c+ GR1+ B220+ or CD8α+ per 10^6 cells
80000
70000
60000
50000
Ova /saline /saline
Ova /saline /ova
Ova /EFD1 /ova
Ova /EFD1 /EFD1 /ova
***
***
Gr1+ B220+
CD8α+

Figure 25

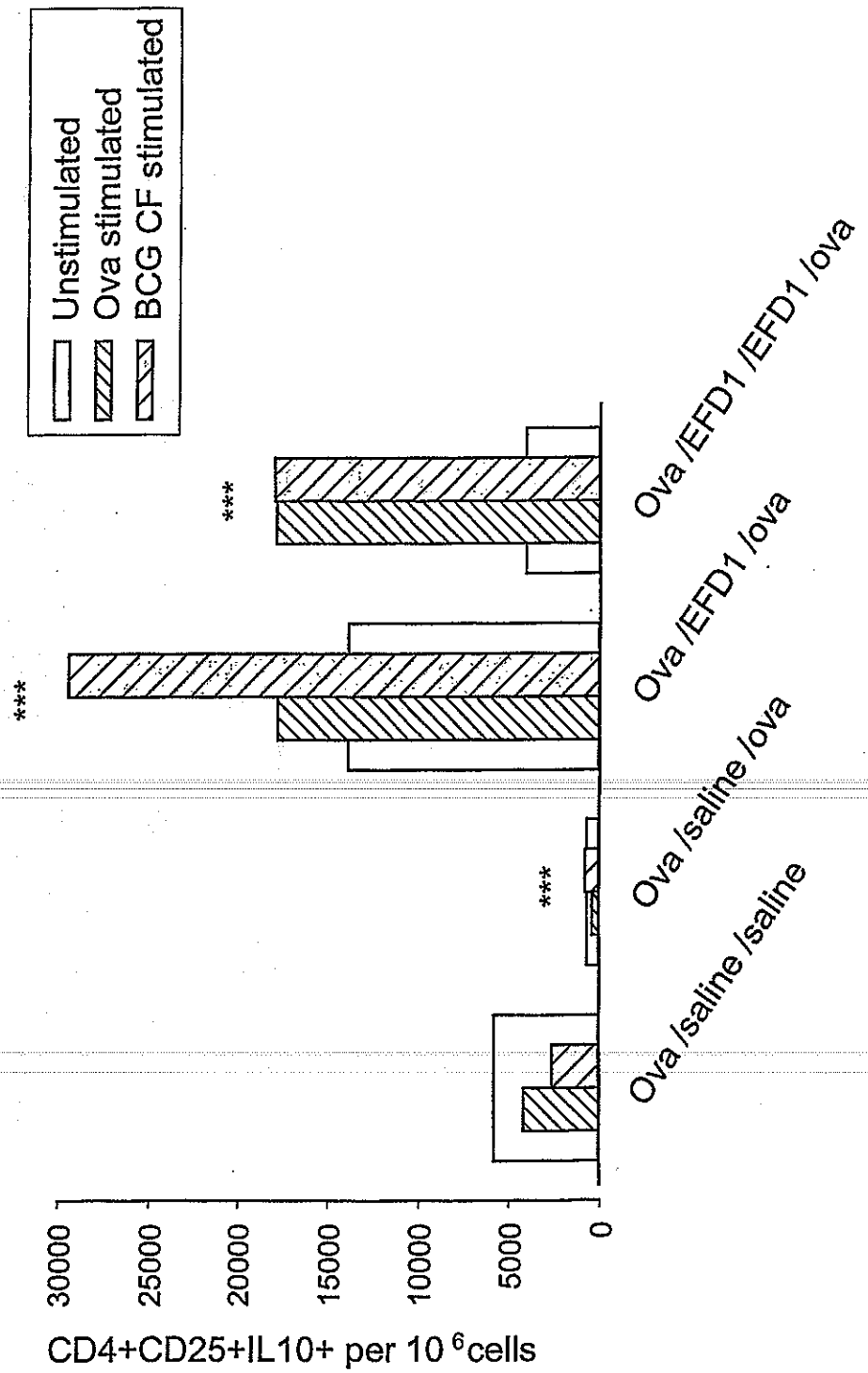

Unstimulated
Ova stimulated
BCG CF stimulated
***
***
***
Ova /EFD1 /EFD1 /ova
Ova /EFD1 /ova
Ova /saline /ova
Ova /saline /saline
30000
25000
20000
15000
10000
5000
0
CD4+CD25+IL10+ per 10 6cells

Figure 26

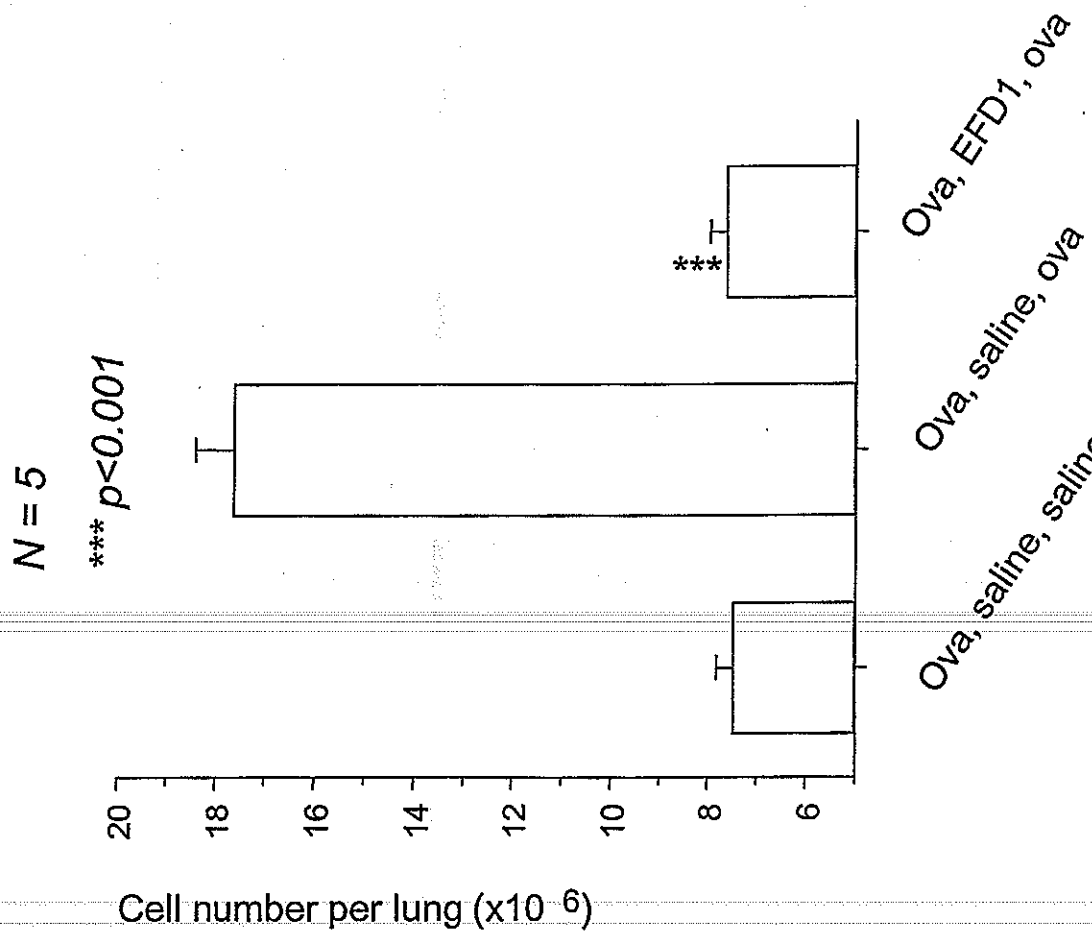

N = 5
*** p<0.001
***
Ova, EFD1, ova
Ova, saline, ova
Ova, saline, saline
20
18
16
14
12
10
8
6
Cell number per lung (x10 6)

Figure 27

Penh
1.75 +/- 0.6
2.60 +/- 0.8
1.80 +/- 0.5
1.60 +/- 0.7
Ova
First challenge
Saline
Challenge
EFD
0
7
14
45
65
96

Figure 28

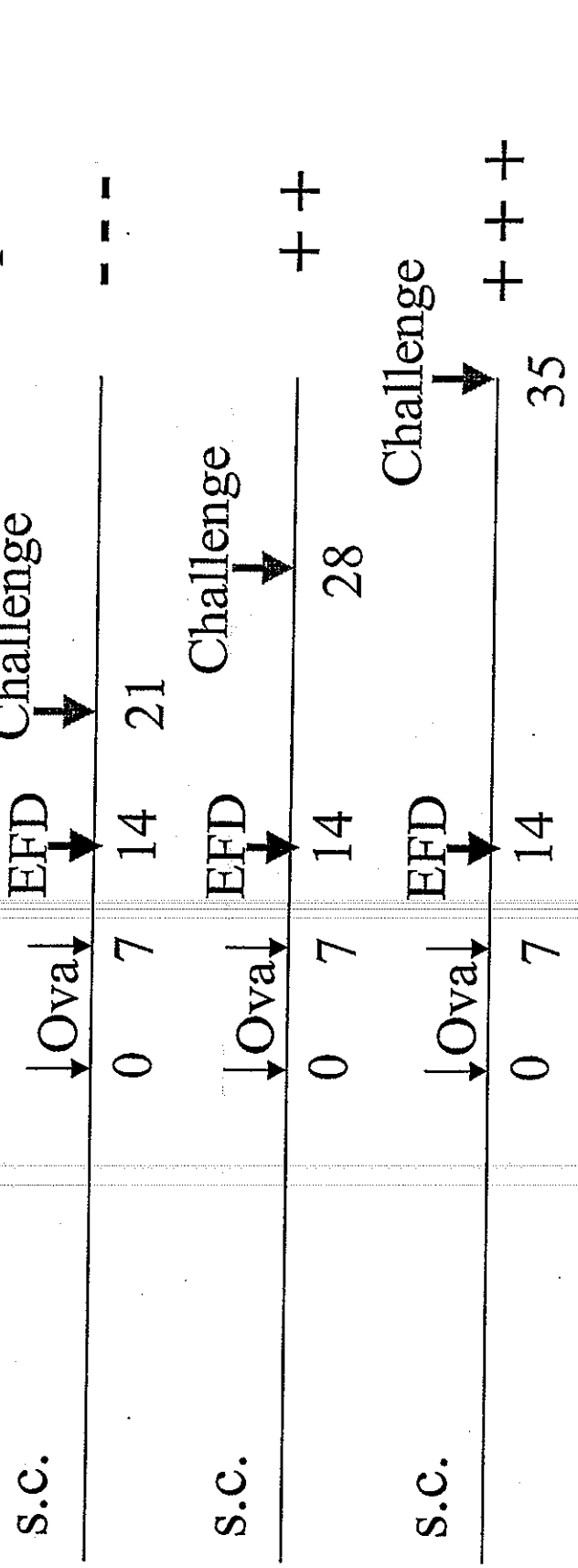

BHR prevention
S.C.
Ova
0
7
EFD
14
Challenge
21
- - -
S.C.
Ova
0
7
EFD
14
Challenge
28
++
S.C.
Ova
0
7
EFD
14
Challenge
35
+++

Figure 29

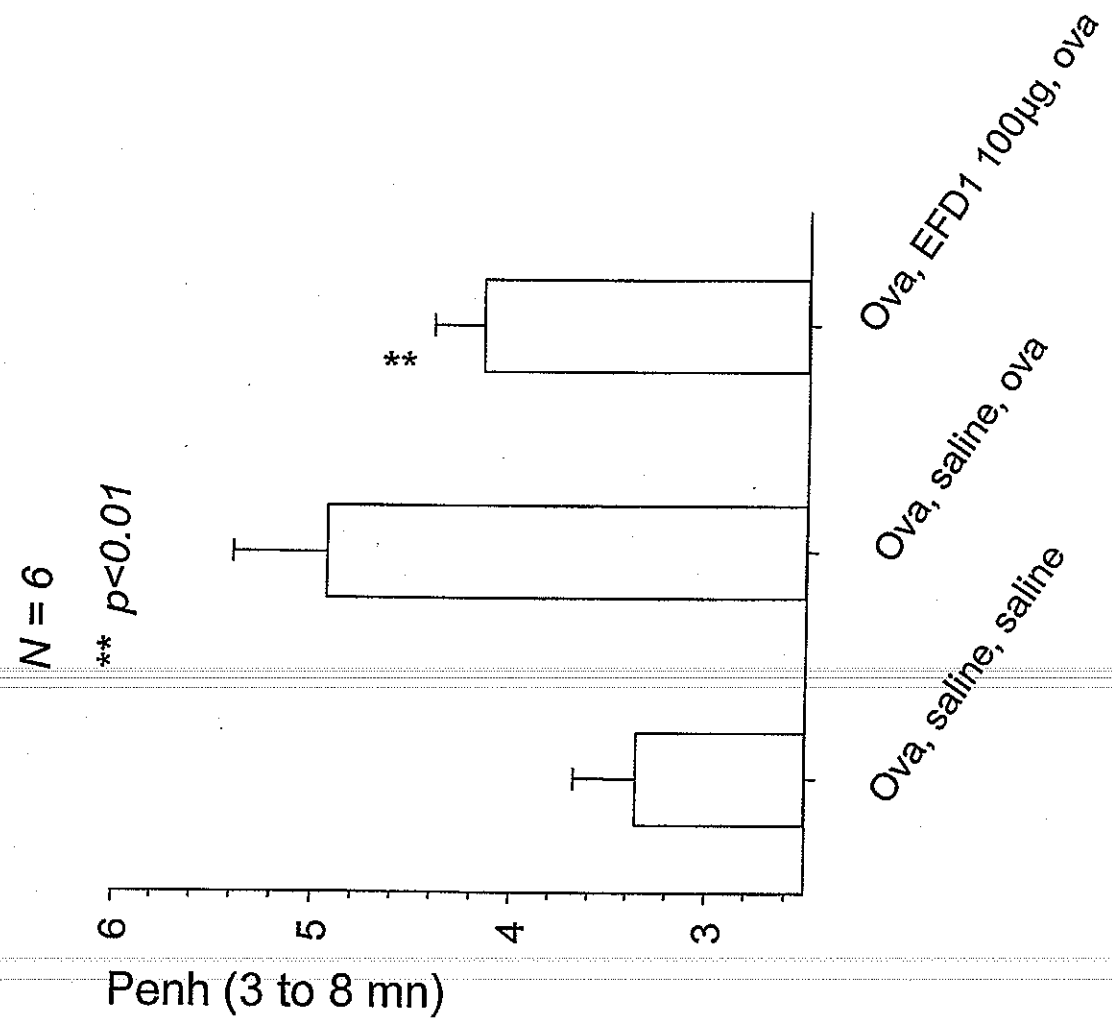

Ova, EFD1 100µg, ova
Ova, saline, ova
Ova, saline, saline
**
N = 6
** p<0.01
6
5
4
3
Penh (3 to 8 mn)

Figure 30