BACTERIAL WORK TO SUPPORT OUR STUDIES

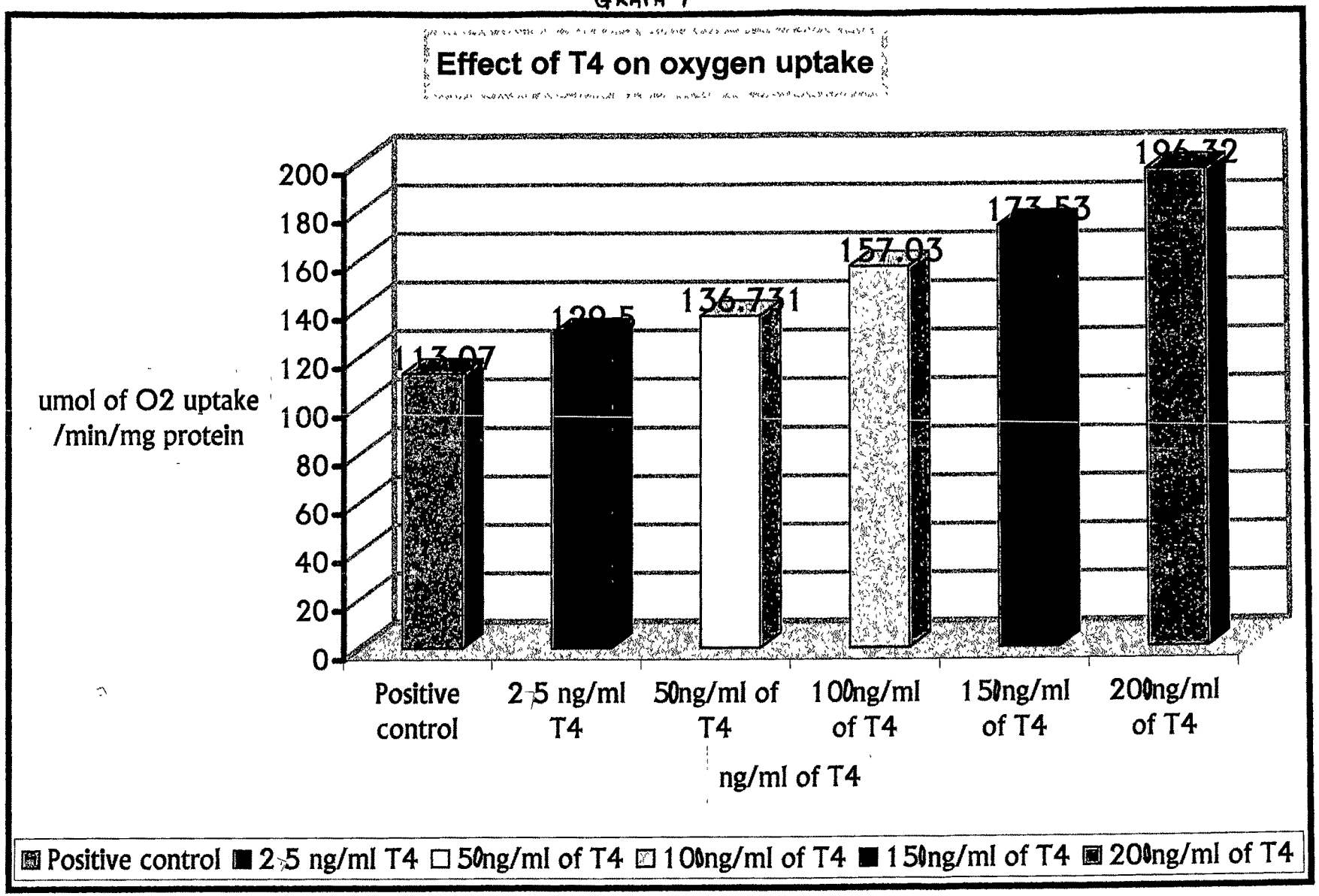
An attempt has been made to study the well established calorigenic effect of thyroid hormone on bacterial growth; *E. coli* (Wild type) was chosen for the study. A minimal Media M9 was used for the study whose composition is given in the Chapter on Materials and Methods.

1. STUDIES ON OXYGEN UPTAKE GRAPH 1 AND GRAPH 2

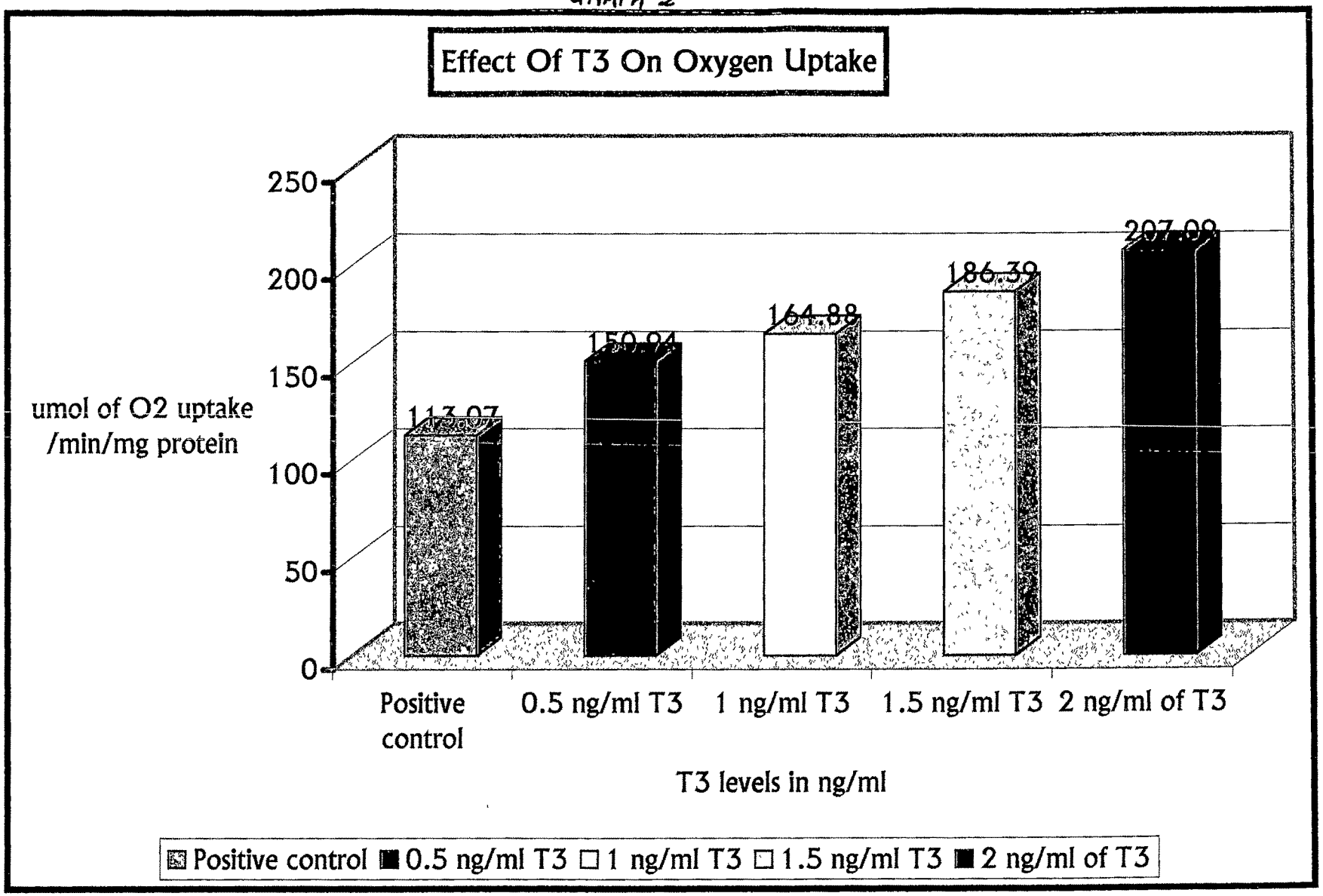
Thyroid hormone acts directly upon the tissue cells by increasing their oxidative processes and oxygen consumption. We studied this aspect in vitro on the cell cultures of Escherichia coli, by adding different concentrations of the thyroid hormone to it and recording the change in the oxygen consumption by the cells by oxygen electrode by GILSON-USA. The procedure is explained in brief below : Minimal medium M9 containing 0.4 gm% glucose. The cells were inoculated for 12 hours in M9-Medium for adaptability. On the next day, 0.5 ml of the cell culture was added to 10 ml of medium M9, in two test tubes and placed on the shaker for a uniform overnight growth for 12 hours.

Next day they were centrifuged, and washed with minimal media M9 without glucose in aseptic conditions, they were washed three times. After the third wash the cells were suspended in 1.5 ml of medium without glucose for 1 hour

GRAPH 1


Effect of T4 on oxygen uptake
umol of O2 uptake /min/mg protein
200
180
160
140
120
100
80
60
40
20
0
113.07
129.5
136.731
157.03
173.53
196.32
Positive control
2.5 ng/ml T4
50ng/ml of T4
100ng/ml of T4
150ng/ml of T4
200ng/ml of T4
ng/ml of T4
Positive control
2.5 ng/ml T4
50ng/ml of T4
100ng/ml of T4
150ng/ml of T4
200ng/ml of T4

GRAPH-2


Effect Of T3 On Oxygen Uptake
umol of O2 uptake /min/mg protein
250
200
150
100
50
0
113.07
150.84
164.88
186.39
207.09
Positive control
0.5 ng/ml T3
1 ng/ml T3
1.5 ng/ml T3
2 ng/ml of T3
T3 levels in ng/ml
Positive control
0.5 ng/ml T3
1 ng/ml T3
1.5 ng/ml T3
2 ng/ml of T3

6. Oxygen uptake by E. coli in presence of 200 ng/ml of T_4 196.32 ± 7.97

7. Oxygen uptake by E. coli in presence of 0.5 ng/ml of T_3 150.94 ± 0.96

8. Oxygen uptake by E. coli in presence of 1.0 ng/ml of T_3 164.88 ± 0.28

9. Oxygen uptake by E. coli in presence of 1.5 ng/ml of T_3 186.39 ± 1.12

10. Oxygen uptake by E. coli in presence of 2.0 ng/ml of T_3 207.09 ± 0.75

2. EFFECT ON GROWTH GRAPH 3 AND GRAPH 4

To study the growth of *E. coli* in presence of thyroid hormone systems of 30 ml containing minimal medium-M9, the cell suspension and the thyroid hormones were prepared.

Method of Study : Growth was followed by nothing the increase in absorbance at 600 nm using a side armed flask.

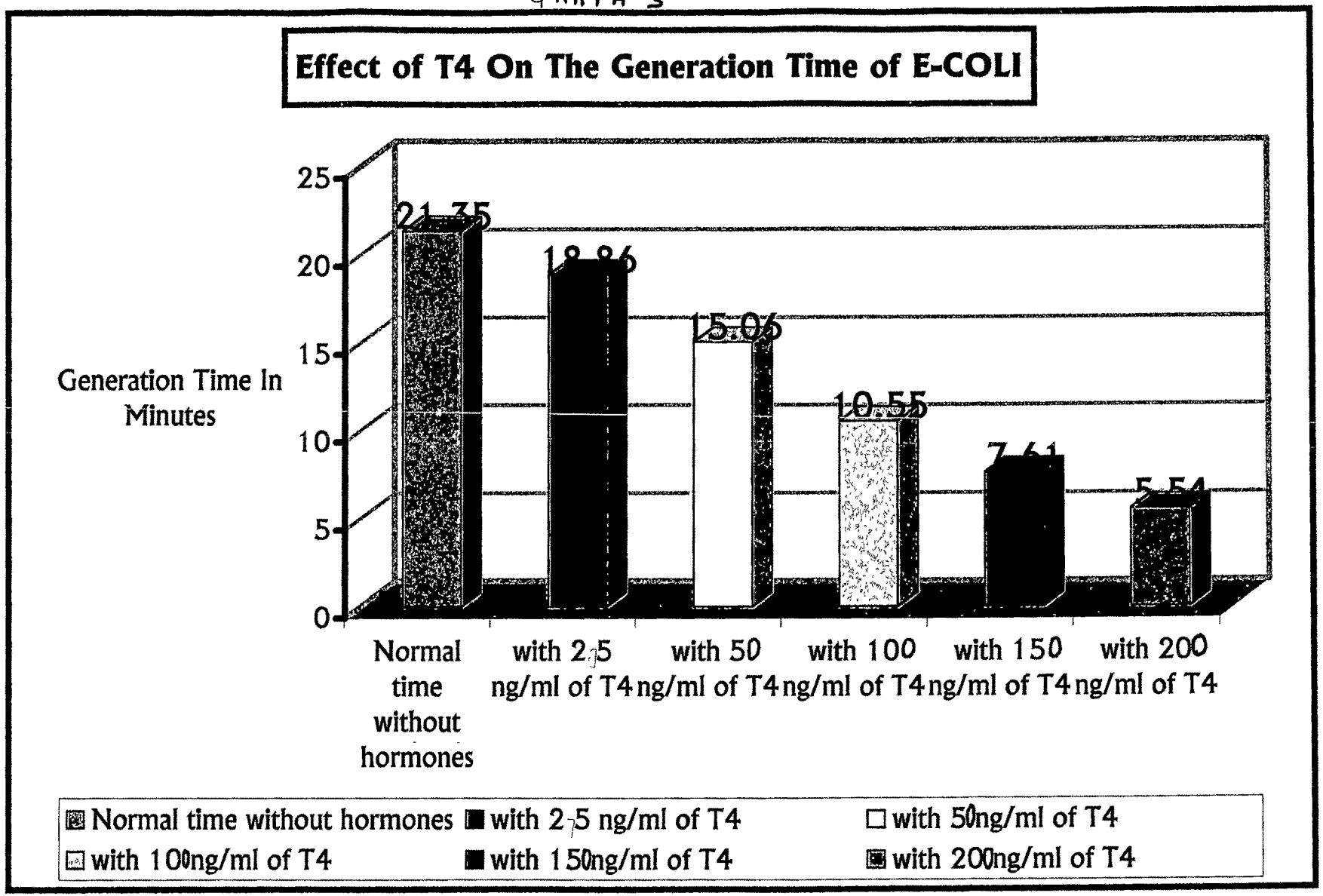
Experimental protocol for thyroxine (stock of thyroxine was prepared using Eltroxine tablets from Glaxo dissolved in sterile distilled water in aseptic condition, the final concentration of which was determined by ELISA).

System-1 consisted of a negative control without cell and hormones.

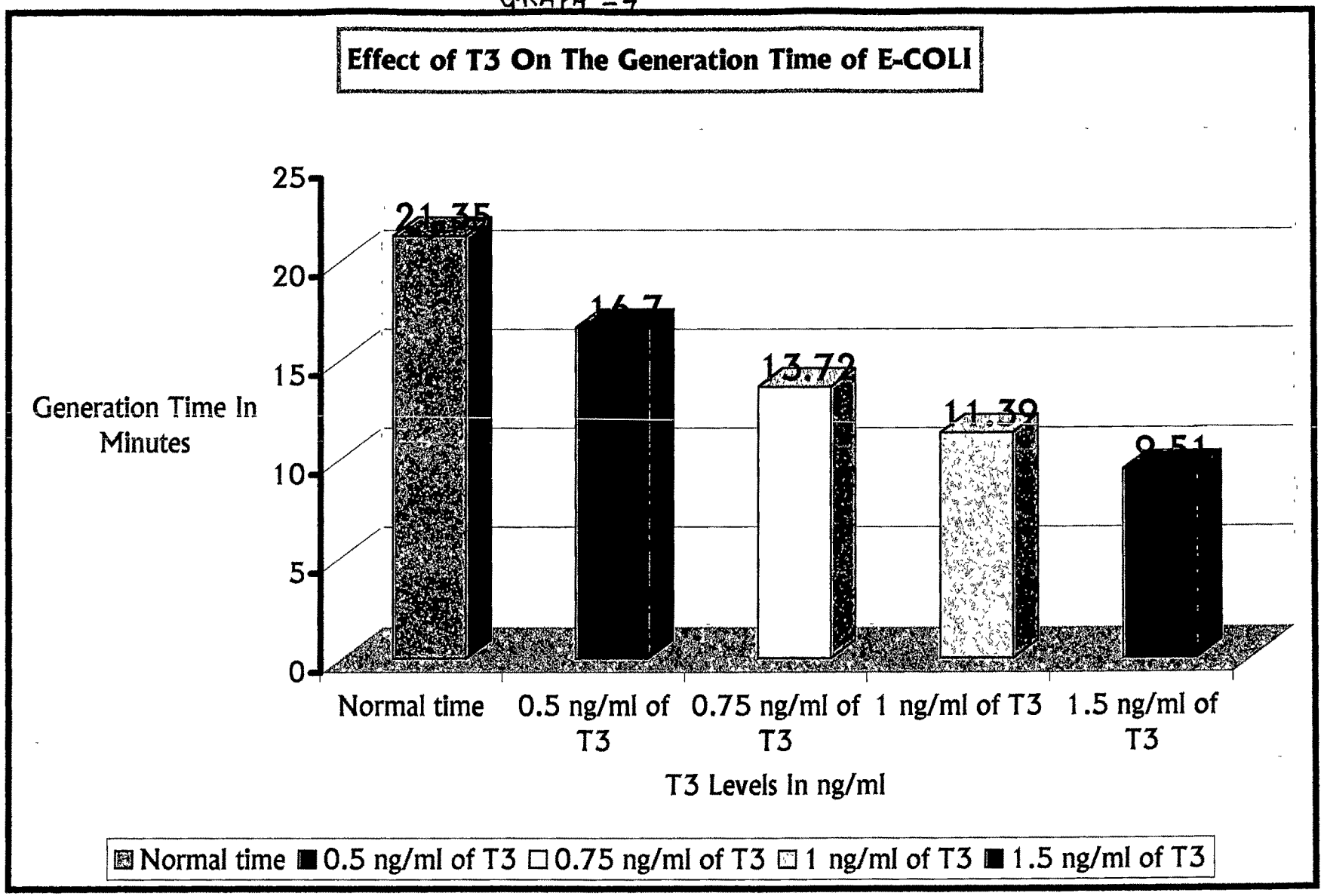
System-2 was a positive control of cells without thyroid hormones.

System-3 to 7 consisting of cells with thyroid hormones in 25 ng/ml, 50 ng/ml, 100 ng/ml, 150 ng/ml and 200 ng/ml of thyroxine.

GRAPH 3


Effect of T4 On The Generation Time of E-COLI
Generation Time In Minutes
25
20
15
10
5
0
21.35
18.86
15.06
10.55
7.61
5.54
Normal time without hormones
with 2.5 ng/ml of T4
with 50 ng/ml of T4
with 100 ng/ml of T4
with 150 ng/ml of T4
with 200 ng/ml of T4
Normal time without hormones
with 2.5 ng/ml of T4
with 50ng/ml of T4
with 100ng/ml of T4
with 150ng/ml of T4
with 200ng/ml of T4

GRAPH - 4


Effect of T3 On The Generation Time of E-COLI
Generation Time In Minutes
25
20
15
10
5
0
21.35
16.7
13.72
11.39
9.51
Normal time
0.5 ng/ml of T3
0.75 ng/ml of T3
1 ng/ml of T3
1.5 ng/ml of T3
T3 Levels In ng/ml
Normal time
0.5 ng/ml of T3
0.75 ng/ml of T3
1 ng/ml of T3
1.5 ng/ml of T3

The effect of triiodothyromine was studied in a similar manner using (0.5 ng/ml T_3, 0.75 ng/ml T_3, 1 ng/ml T_3 and 1.5 ng/ml T_3) obtained from Diagnostic System Lab USA, the final concentration of which was determined by standard ELISA.

Results are as follows :

Generation time for Normal E. coli	→	21.35 min ± 0.72
Generation time of E. coli with 25 ng/ml of T_4	→	18.86 min ± 0.65
Generation time of E. coli with 50 ng/ml of T_4	→	15.06 min ± 0.83
Generation time of E. coli with 100 ng/ml of T_4	→	10.55 min ± 0.54
Generation time of E. coli with 150 ng/ml of T_4	→	7.61 min ± 0.54
Generation time of E. coli with 200 ng/ml of T_4	→	5.54 min ± 0.89
Generation time of E. coli with 0.5 ng/ml of T_3	→	16.7 min ± 0.62
Generation time of E. coli with 0.75 ng/ml of T_3	→	13.72 min ± 0.35
Generation time of E. coli with 1 ng/ml of T_3	→	11.39 min ± 0.64
Generation time of E. coli with 1.5 ng/ml of T_3	→	9.51 min ± 0.55

3. ENZYME STUDIES GRAPH 5

The enzyme responsible for the conversion of the T_4 to T_3 is iodothyronine-5'-deiodinase, which exists as two distinct isoenzymes that are differentially expressed and regulated in peripheral tissues. Whether or not this mechanism,

GRAPH 5

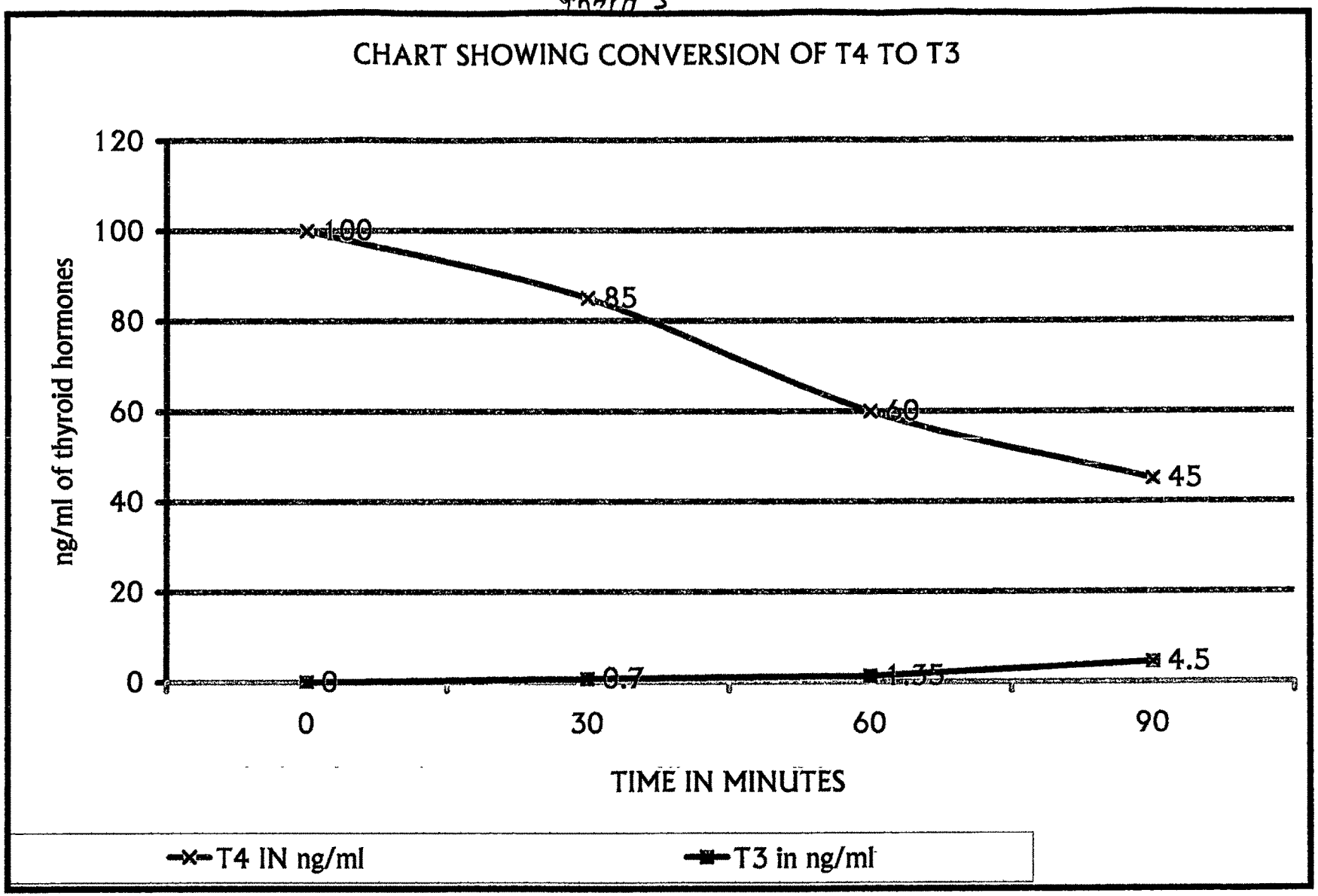


CHART SHOWING CONVERSION OF T4 TO T3
ng/ml of thyroid hormones
120
100
80
60
40
20
0
100
85
60
45
0
0.7
1.35
4.5
0
30
60
90
TIME IN MINUTES
T4 IN ng/ml
T3 in ng/ml

may be functioning in the bacterial system or the presence of a different pathway of thyroxine (T_4) breakdown to T_3 needs a lot of research to be done.

To study the conversion of T_4 to T_3 in the bacterial system, Tris-HCl buffer of pH 7.4 was prepared along with minimal media M9 (100 ml) with glucose. The system consisted of 100 ng/dl of thyroxine in 30 ml of medium M9 with glucose with 0.3 ml of 1% inoculum of *E. coli*. The initial concentration of T_4 and T_3 was noted by taking 1.5 ml of the above system, centrifuging it, re-suspending the pellet in 0.5 ml of Tris-HCl buffer and sonicating it.

After sonicating the eppendrof tubes were centrifuged, the pellet consisting of debris of broken cells was discarded and the supernatant was preserved for the estimation of T_4 and T_3 by ELISA. The similar procedure was carried out at the interval of 30 minutes, 60 minutes and 90 minutes and the supernatant utilized for the estimation of T_4 and T_3.

Result

Time	Amount of T_4	Amount of T_3 formed
0 minute	100 ng/ml	0 ng/ml
30 minutes	85 ng/ml	0.7 ng/ml
60 minutes	60 ng/ml	1.35 ng/ml
90 minutes	45 ng/ml	4.5 ng/ml

Thyroid hormones have a stimulatory effect on metabolism in animals, and its stimulatory effect on M. tuberculous and Mycobacterium Leprae has been reported by SK Biswas (1975, 1989). The results of the effect of thyroid hormone on the growth of *E. coli* indicate the T_3 has a greater stimulatory effect than T_4, in a ratio of 50:1. Even the oxygen consumption is greater even with lower concentrations of T_3 than T_4.