RESULTS

R E S U L T S

EXPERIMENT I.

CELL KINETICS.

According to the estimates obtained by statistical analysis, the blood cultures of the 15 donors were classified into two groups; one containing the male donors (8 cases) and the other containing the female ones (7 cases).

Group 1 (Male Donors):-

Control Cultures.

At a calcium ion concentration of 50 µg/ml., (control cultures), there was a sharp decline of the cell density after 24 hour-incubation period, followed by gradual increase thenceafter but the initial cell density (200×10^3 cells/ml.) was not restored even after 96 hours of incubation. The population variances of these cultures (σ^2) were estimated by 7.143, 31.697, 35.714 and 81.697 after 24, 48, 72 and 96 hours of incubation respectively.

Model A.

In this experimental model, phytohaemagglutinin-M (PHA-M) was added in a constant concentration (40 µg/ml.) on an already adjusted calcium ion concentration.

The quantitation estimates of the individual donors were shown in tables 1-8 and the mean values were presented in table 9. A comparative plot for the mean values of control cultures and those containing ascending concentrations of calcium ion in model A cultures was represented by fig.1.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control *		200	100	110	120	160	682
Model A	70	200	120	160	205	240	432
	90	200	80	90	90	80	790
	110	200	40	25	10	5	21
	130	200	25	5	0	0	0
Model B	70	200	105	120	155	180	465
	90	200	90	100	100	95	799
	110	200	55	45	20	10	16
	130	200	30	10	0	0	0
Model C	70	200	110	145	180	215	528
	90	200	105	110	110	100	782
	110	200	65	50	30	15	11
	130	200	40	20	5	0	0
Model D	70	200	120	165	210	250	435
	90	200	115	125	120	105	647
	110	200	75	60	45	25	5
	130	200	40	25	10	0	0

Table 1 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 1 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control *		200	95	100	105	135	575
Model A	70	200	120	155	200	240	423
	90	200	75	90	90	80	787
	110	200	50	35	20	0	0
	130	200	20	0	0	0	0
Model B	70	200	110	120	160	190	472
	90	200	85	100	100	90	796
	110	200	60	40	25	10	24
	130	200	25	5	0	0	0
Model C	70	200	110	150	185	225	501
	90	200	90	105	110	100	791
	110	200	65	45	30	20	19
	130	200	30	15	10	0	0
Model D	70	200	120	165	205	250	431
	90	200	100	105	100	90	643
	110	200	70	45	35	20	17
	130	200	40	20	10	5	10

Table 2 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 2 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control *		200	95	100	110	150	639
Model A	70	200	125	165	205	250	441
	90	200	70	85	90	80	793
	110	200	45	30	20	5	23
	130	200	25	5	0	0	0
Model B	70	200	115	125	155	190	480
	90	200	80	100	95	80	789
	110	200	55	45	30	10	19
	130	200	25	10	5	0	0
Model C	70	200	120	145	185	220	512
	90	200	90	105	105	95	799
	110	200	60	50	35	10	21
	130	200	35	20	10	10	8
Model D	70	200	125	170	210	260	446
	90	200	105	105	100	90	652
	110	200	65	45	30	15	15
	130	200	45	25	15	10	9

Table 3 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 3 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I). Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	100	115	120	155	647
Model A	70	200	120	165	205	250	436
	90	200	85	95	90	80	801
	110	200	45	30	15	5	25
	130	200	20	0	0	0	0
Model B	70	200	105	115	155	185	469
	90	200	95	100	100	95	812
	110	200	55	40	25	10	17
	130	200	25	10	0	0	0
Model C	70	200	115	150	190	225	517
	90	200	110	115	110	100	798
	110	200	70	50	35	20	11
	130	200	30	15	0	0	0
Model D	70	200	120	160	210	245	429
	90	200	115	125	110	105	649
	110	200	75	50	35	25	7
	130	200	45	25	10	0	0

Table 4 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 4 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	95	105	115	140	589
Model A	70	200	125	165	210	255	443
	90	200	75	90	85	85	800
	110	200	40	20	5	0	0
	130	200	25	10	5	0	0
Model B	70	200	110	120	145	185	459
	90	200	85	95	100	90	806
	110	200	55	30	15	10	15
	130	200	30	15	10	0	0
Model C	70	200	115	150	190	220	531
	90	200	105	115	110	100	803
	110	200	65	45	35	20	10
	130	200	40	25	20	10	6
Model D	70	200	125	160	215	255	440
	90	200	115	130	125	105	659
	110	200	75	55	35	25	8
	130	200	40	30	25	10	8

Table 5 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 5 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control * = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control *		200	100	105	120	160	702
Model A	70	200	115	155	195	235	398
	90	200	85	95	90	90	806
	110	200	45	30	20	10	20
	130	200	20	10	5	0	0
Model B	70	200	95	115	145	180	453
	90	200	95	105	100	100	813
	110	200	55	40	25	10	18
	130	200	30	10	10	0	0
Model C	70	200	110	145	180	200	499
	90	200	105	115	105	100	804
	110	200	60	45	30	15	14
	130	200	40	20	15	5	9
Model D	70	200	120	170	200	245	405
	90	200	115	115	110	100	654
	110	200	70	50	35	20	9
	130	200	40	20	10	0	0

Table 6 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 6 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control * = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control *		200	100	110	120	155	661
Model A	70	200	115	150	190	235	407
	90	200	80	85	85	80	795
	110	200	45	30	20	10	21
	130	200	20	10	5	5	18
Model B	70	200	95	110	145	175	458
	90	200	90	95	100	90	803
	110	200	55	40	30	10	16
	130	200	30	15	10	0	0
Model C	70	200	110	140	175	200	503
	90	200	105	110	100	95	800
	110	200	70	55	35	20	10
	130	200	30	20	10	5	8
Model D	70	200	120	160	200	245	412
	90	200	115	125	115	105	650
	110	200	80	65	45	25	8
	130	200	45	30	15	5	11

Table 7 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 7 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	95	100	110	150	646
Model A	70	200	125	170	215	260	450
	90	200	85	95	90	90	799
	110	200	45	30	15	15	19
	130	200	25	10	5	0	0
Model B	70	200	110	130	170	195	467
	90	200	90	100	100	95	811
	110	200	60	45	20	15	16
	130	200	35	15	5	0	0
Model C	70	200	120	150	195	225	519
	90	200	105	115	110	100	807
	110	200	70	50	35	15	12
	130	200	45	25	10	10	10
Model D	70	200	130	170	210	260	442
	90	200	120	130	125	110	652
	110	200	80	60	40	20	10
	130	200	45	30	15	10	12

Table 8 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 8 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	97.5	105.6	115.0	150.6	642.6
Model A	70	200	120.6	160.6	203.0	245.6	428.8
	90	200	79.6	90.6	88.8	83.0	796.4
	110	200	44.4	28.8	15.6	6.3	16.1
	130	200	22.5	6.3	2.5	0.6	2.3
Model B	70	200	105.6	119.0	153.8	185.0	465.4
	90	200	88.8	101.9	99.4	91.9	803.6
	110	200	56.3	40.6	23.8	10.6	17.6
	130	200	28.8	11.3	5.0	0.0	0.0
Model C	70	200	113.8	146.9	185.0	216.3	513.8
	90	200	101.9	111.3	107.5	98.8	798.0
	110	200	65.6	48.8	33.0	16.9	13.5
	130	200	36.3	20.0	10.0	5.0	5.1
Model D	70	200	122.5	165.0	207.5	251.3	430.0
	90	200	112.5	120.0	113.0	101.3	650.8
	110	200	73.8	53.8	37.5	21.9	9.9
	130	200	42.5	25.6	13.8	5.0	6.3

Table 9 :- Showing the mean values of the cell kinetics and the transformation score in different culture models at different calcium ion concentrations in the male group (Experiment I). Control* = 50 µg/ml.

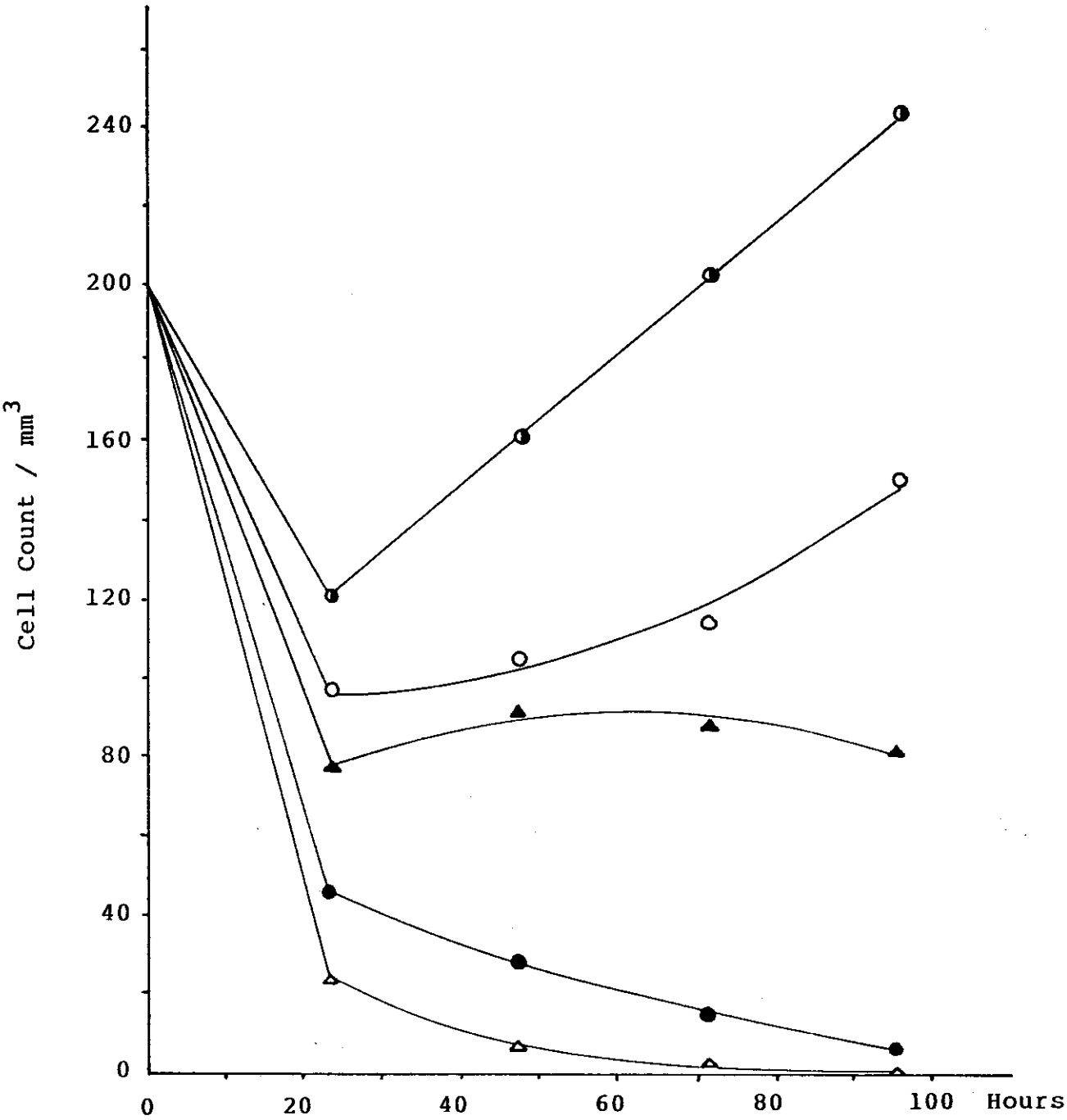

Fig.1:- Comparing the mean values of cell kinetics in the control and experimental cultures of the male donors in model A (Experiment I.). O——O, control cultures; ◑——◑, 70 µg; ▲——▲ ,90 µg; ●——●, 110 µg and △——△ , 130 µg Ca^{++}/ml.

At a calcium ion concentration of 70 µg/ml., the 24 hours' decline of the cell density was also observed but not to the same extent exhibited in control cultures. Cell growth and consequently higher cell density was observed after 48 hours and the initial count was restored around the 72 hour-incubation period. Higher cell densities were observed after 96 hours of incubation. The population variances (σ^2) of these cultures were calculated and found to be 17.411, 45.983, 63.857 and 88.84 after 24, 48, 72 and 96 hour-incubation periods respectively.

At the concentration of 90 µg. of calcium ions per ml. of culture, a much more decline of the cell density was shown after 24 hours and the quantitation values of the cell population were fairly stable along the remaining culture period with a blunt apex between the 48 and the 72 hour-incubation periods. The population variances (σ^2) of these cultures were represented by 31.754, 15.697, 1.337 and 21.00 after 24, 48, 72 and 96 hour-incubation periods respectively.

At calcium ion concentrations of 110 and 130 µg/ml. of culture, a severe decline was noted after 24 hours even reaching one tenth of the initial cell density at the latter concentration. A gradual decrease of the cell density was observed and occasionally reaching zero after 96 hours at 110 µg/ml. concentration and usually reaching zero at 130 µg/ml. concentration. At the latter concentration, complete cell death was observed after 72 hours in 4 cases (50%) and after 48 hours in 2 cases (25%). The population variances of these cultures (σ^2) were calculated and found to be 10.269, 19.623, 31.697 and 26.749 after 24, 48, 72 and 96 hour-incubation periods respectively for the 110 µg/ml. concentration and 7.143, 19.589, 7.143 and 3.126 after the corresponding hours of incubation for

the 130 µg/ml. concentration.

At calcium ion concentration of 150 µg/ml. of culture (equivalent to blood level of 30 mg%), complete cell death was observed after 24 hour-incubation period in all cultures of all donors.

Model B.

Herein, PHA-M was added to standard cultures (50 µg of Ca^{++} per ml. of culture) in the same concentration (40 µg/ml.) 5 minutes before elevating the Ca^{++} level to 70, 90, 110, 130 or 150 µg/ml.

The quantitation estimates of the individual donors were shown in tables 1-8 and the mean values in table 9. A comparative plot for the mean values of control cultures and those containing ascending concentrations of calcium ion (70-130 µg/ml.) in model B cultures was represented by fig.2.

At the concentration of 70 µg/ml., the first 24 hours' decline was also observed but the cell count was still higher than that in control cultures. A gradual expansion of the cell population was then noted reaching the maximum level at 96 hours of incubation. The population variances (σ^2) of these cultures were estimated by 29.983, 39.00, 76.789 and 42.857 after 24, 48, 72 and 96 hour-incubation periods respectively.

At a calcium ion concentration of 90 µg/ml., the cell count was more or less stable but lower than the control values. The cell count was much nearer to the control value at 48 hour-incubation period and the two curves were divergent thenceforward with prolonging the incubation period. The population variances of these cultures (σ^2) were calculated and found to be

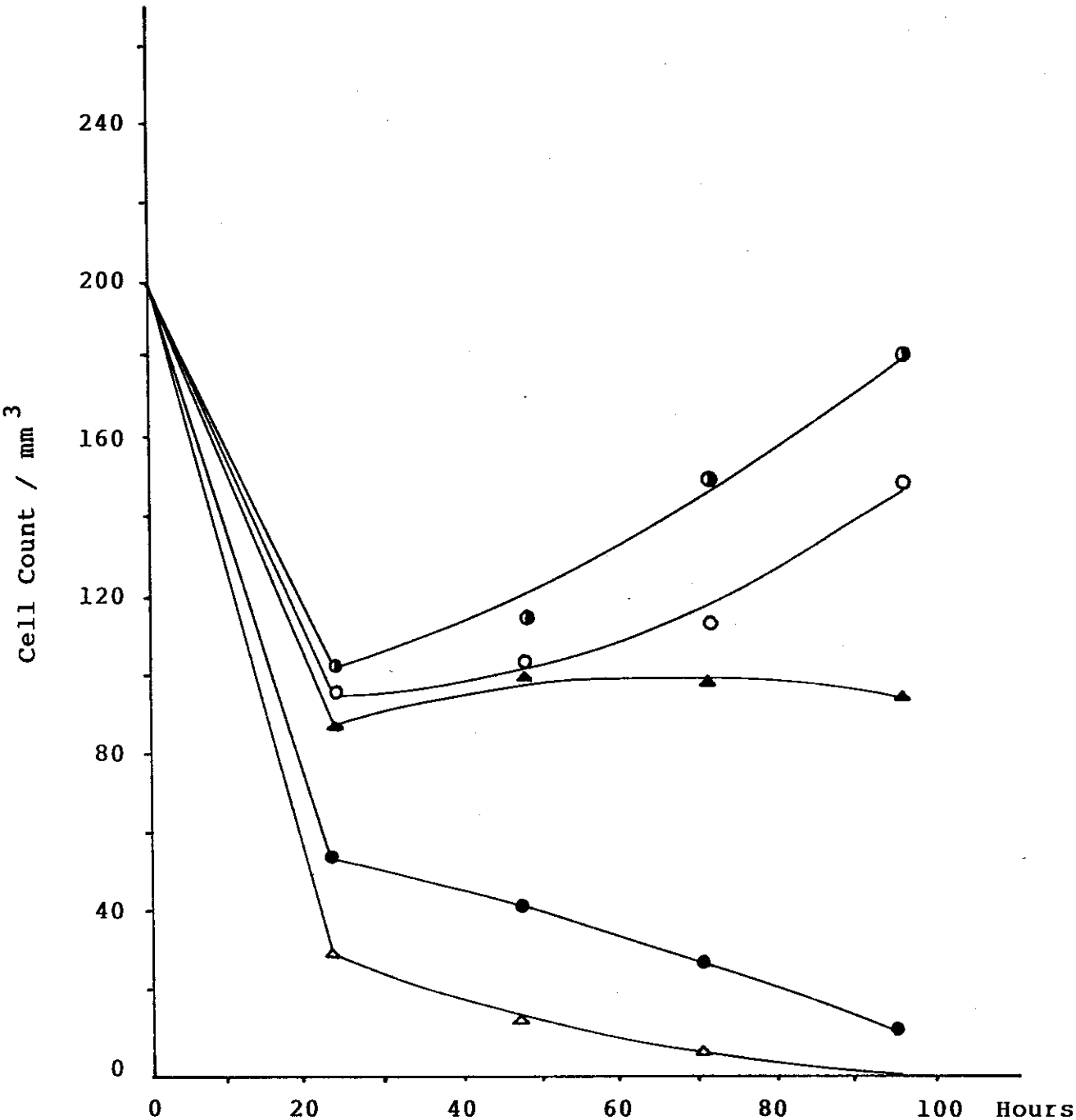

Fig.2:- Comparing the mean values of cell kinetics in the control and experimental cultures of the male donors in model B (Experiment I.). O——O, control cultures; ◐——◐, 70 µg; ▲——▲ ,90 µg; ●——●, 110 µg and △——△ , 130 µg Ca^{++}/ml.

26.789, 17.554, 3.126 and 35.269 after 24, 48, 72 and 96 hours of incubation respectively.

At calcium ion concentrations of 110 and 130 µg/ml., there was a continuous and gradual decline of the cell density with prolonging the period of incubation. At the latter concentration, there was complete cell death as early as 72 hour-incubation period in 3 cases (37.5%) and complete death in all cases after 96 hours of incubation. The population variances of these cultures (σ^2) were estimated by 5.36, 24.554, 26.789 and 3.126 for the 110 µg/ml. concentration and 12.503, 12.503, 21.429 and zero for the 130 µg/ml. concentration; after 24, 48, 72 and 96 hours of incubation respectively.

At calcium ion concentration of 150 µg/ml. of culture (equivalent to blood level of 30 mg%), complete cell death was observed after 24 hour-incubation period in cultures of all donors.

Model C.

Herein, the calcium ion level was adjusted -to fit the tested ascending values- one hour after the addition of PHA-M.

The cell counts of the individual donors were shown in tables 1-8 and the mean values in table 9. The plot presented in fig.3 was drawn to compare the mean values of control cultures and those containing the tested calcium ion concentrations (70-130 µg/ml.) in model C cultures.

At a calcium ion concentration of 70 µg/ml., the cell count was decreased after 24 hours of incubation and followed by a gradual increase thenceafter. The cell density of the initial inoculum was restored between

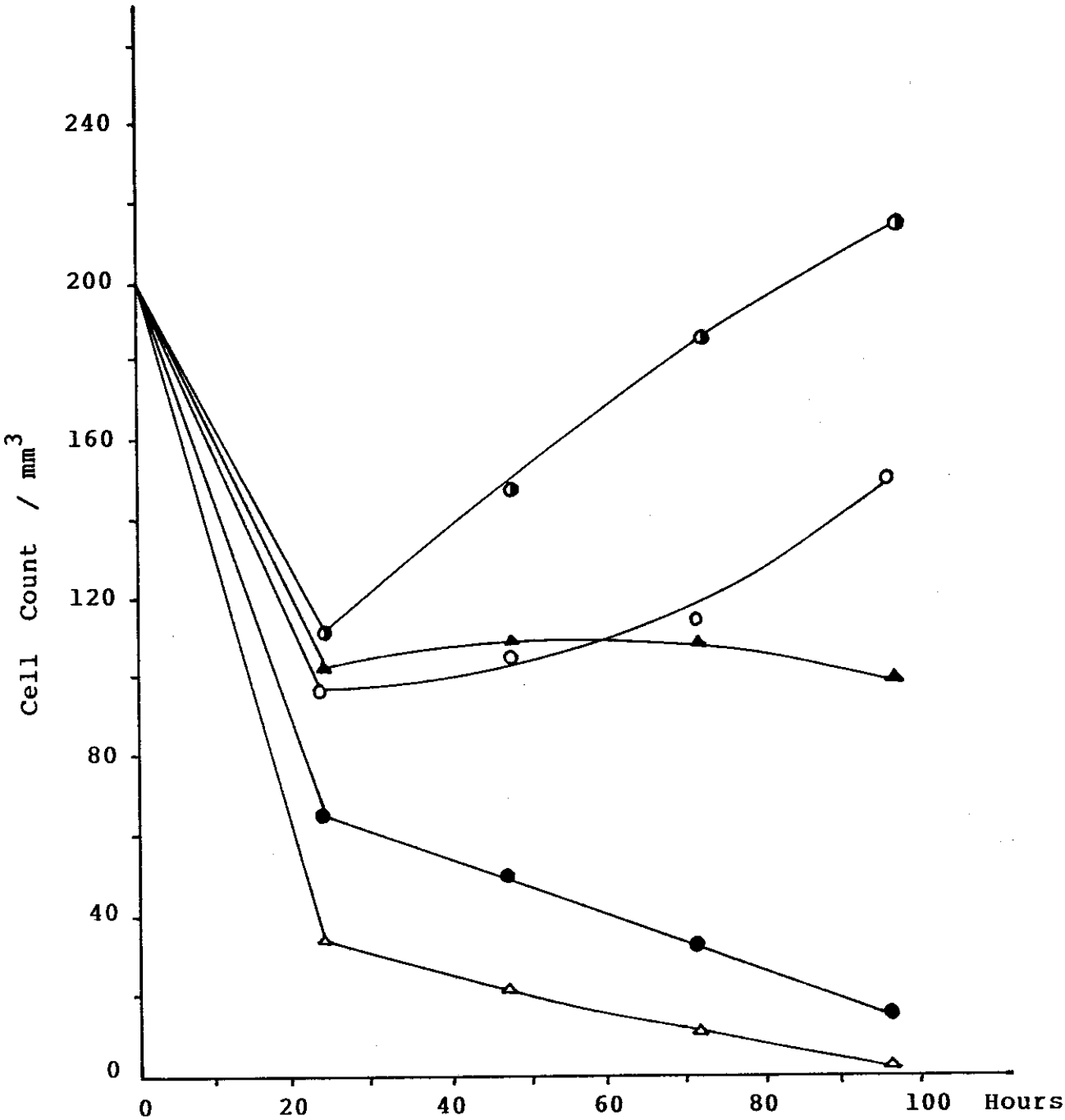

Fig.3:- Comparing the mean values of cell kinetics in the control and experimental cultures of the male donors in model C (Experiment I.). O——O, control cultures; ◐——◐, 70 µg; ▲——▲ ,90 µg; ●——●, 110 µg and △——△ , 130 µg Ca^{++}/ml.

72 and 96 hour-incubation periods. The quantitation values obtained in this Ca^{++} concentration were higher than the corresponding values of the control cultures in all incubation periods. The population variances of these cultures (σ^2) were calculated and found to be 19.646, 13.84, 42.857 and 112.503 after 24, 48, 72 and 96 hours of incubation respectively.

At the concentration of 90 µg/ml., there was a sharp decline of the cell count reaching about 50% of the initial inoculum followed by a more or less stable cell count up to 72 hours of incubation after which a second decline was noted. The population variances of these cultures (σ^2) were estimated by 56.697, 19.646, 14.286 and 5.36 after 24, 48, 72 and 96 hour-incubation periods respectively.

At calcium ion concentrations of 110 and 130 µg/ml., the sudden decline after 24 hours of incubation was noted and followed by gradual decrease of the cell density with prolonging the incubation period. The quantitation estimates never reached zero at 110 µg/ml. concentration whereas they reached zero in 3 cases (37.5%) at the concentration value of 130 µg/ml. The population variances (σ^2) of these cultures were estimated by 17.411, 12.503, 6.714 and 13.84 for the 110 µg/ml. concentration and 33.931, 14.286, 35.714 and 21.429 for the 130 µg/ml. concentration; after 24, 48, 72 and 96 hours of incubation respectively.

At calcium ion concentration of 150 µg/ml. of culture (equivalent to blood level of 30 mg%), complete cell death was observed after 24 hour-incubation period in cultures of all donors.

Model D.

In this experimental model, PHA-M was added to the standard cultures 6 hours prior to the adjustment of

Ca^{++} concentration.

The quantitation estimates of the individual donors were shown in tables 1-8 and the mean values were presented in table 9. A comparative plot for the mean values of control cultures and those containing the ascending concentrations of calcium ion (70-130 µg/ml.) in model D cultures was represented by fig.4.

At the 70 µg/ml. concentration , the sharp 24 hours' decline was noticed, followed by a gradual recovery of the cell density and the count of the initial inoculum was restored nearly at 72 hour-incubation period. The mean values of the quantitation estimates were very near to their corresponding values calculated for model A. The population variances (σ^2) of these cultures were estimated by 14.286, 21.429, 28.572 and 41.074 after 24, 48, 72 and 96 hours of incubation respectively.

At a concentration of 90 µg Ca^{++} per ml. of culture, the 24 hours' decline was quantitatively less than the control cultures' decline. However, there was no gradually-increasing recovery and the cell density was fairly stable along the first 72 hours of incubation with a second gradual decline thenceafter. The cell density coincided with that of the control at 72 hours of incubation. The population variances (σ^2) of these cultures were calculated and found to be 42.858, 107.145, 99.571 and 52.503 after 24, 48, 72 and 96 hour-incubation periods respectively.

At calcium ion concentrations of 110 and 130 µg/ml., there was a sudden sharp decline of the cultures' cellularity after 24 hours of incubation followed by persistent gradual deterioration of the quantitation estimates. At the latter concentration (130 µg/ml.),

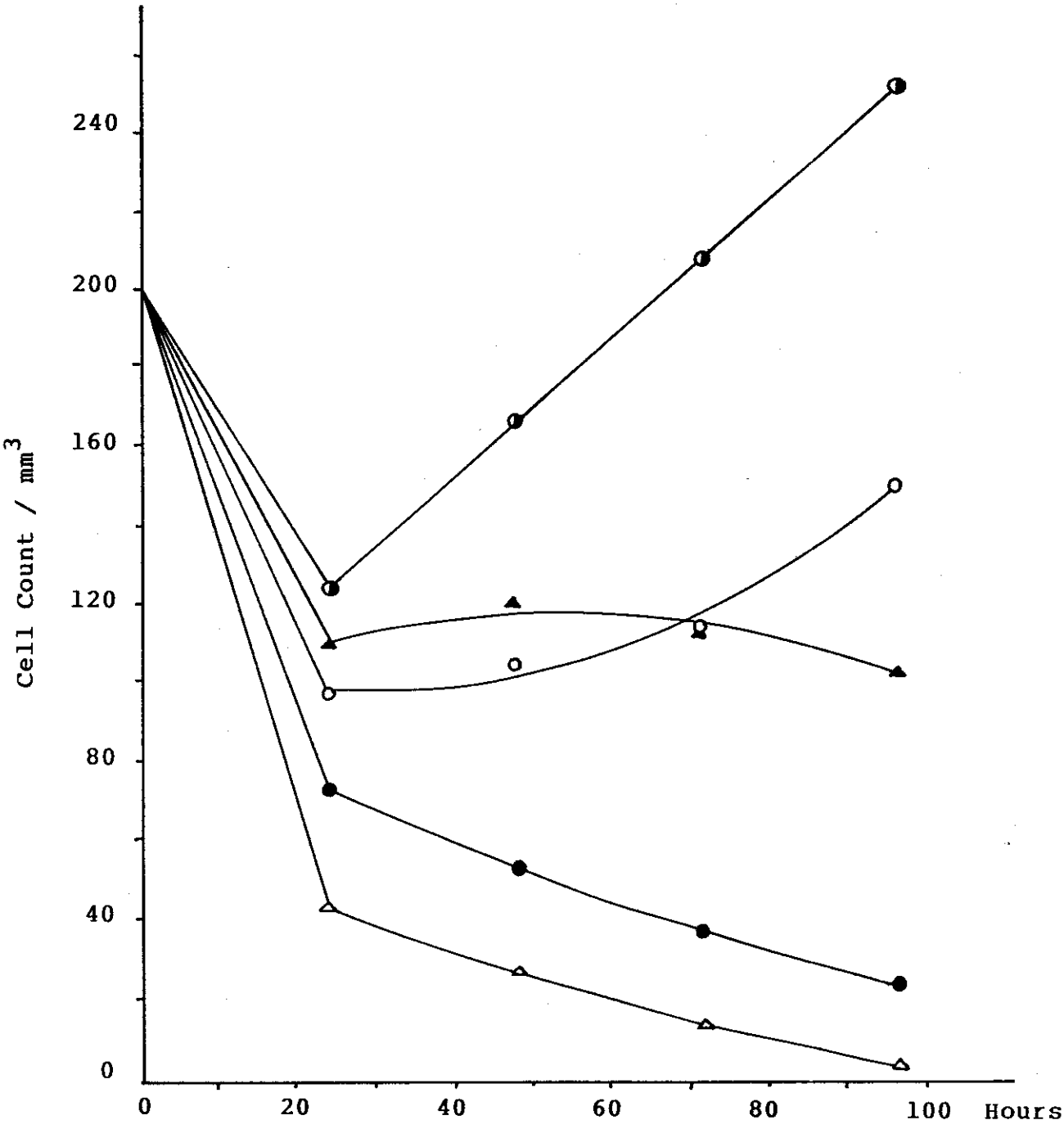

Fig.4:- Comparing the mean values of cell kinetics in the control and experimental cultures of the male donors in model D (Experiment I.). O——O, control cultures; ◐——◐, 70 µg; ▲——▲ ,90 µg; ●——●, 110 µg and △——△ , 130 µg Ca^{++}/ml.

the cultures of 3 donors (37.5%) showed no single viable cell after 96 hour-incubation period. The population variances (σ^2) of the cultures at these concentrations were estimated by 26.789, 55.36, 28.572 and 13.84 for the 110 µg/ml. concentration and 7.143, 17.411, 26.789 and 21.429 for the 130 µg/ml. concentration; after 24, 48, 72 and 96 hours of incubation respectively.

At calcium ion concentration of 150 µg/ml. of culture (equivalent to blood level of 30 mg%), complete cell death was observed after 24 hour-incubation period in cultures of all donors.

Group 2 (Female Donors):-

In this part of the study, the cell behaviour was analogous to that observed in the male group with the exception that the cell counts of the experimental cultures were usually lower than their correspondings in group 1.

Control Cultures.

In these cultures, there was an initial decline of cultures' cellularity. However, the estimates were higher than their correspondings in the male group. Prolongation of the incubation period resulted in restoration of the cell density of the initial inoculum between 72 and 96 hour-incubation periods. The cell count was steadily increasing and exceeded the value of the initial cultures at 96 hours of incubation. The population variances (σ^2) of these cultures were estimated by 14.287, 40.50, 47.62 and 105.953 after 24, 48, 72 and 96 hours of incubation respectively.

Model A.

The quantitation estimates of the individual donors

were shown in tables 10-16 and the mean values in table 17. Fig.5 was drawn to compare the mean values of cellular kinetics after different hour-incubation periods in the control group as well as in experimental cultures of model A.

In this experimental model, the 24 hours' decline was also exhibited in all experimental cultures. However, the quantitation estimates were always higher in the control cultures. The maximal lytic effect on the cells was observed with calcium ion concentration of 150 µg/ml. at which the quantitation value reached zero in all cultures of all donors. However, at calcium ion concentration of 130 µg/ml., complete cell death was observed in about 6 out of the 7 tested cases (85.7%).

Prolongation of the incubation period resulted in a progressive increase of the cell density in cultures subjected to Ca^{++} concentration of 70 µg/ml. but never reaching the initial value. However, with 90 µg/ml. concentration, the cell count was fairly stable and a blunt peak of the curve was noticed between 48 and 72 hours of incubation. In contrast, a dramatic deterioration of the cultures' cellularity was noted with higher concentrations (110, 130 and 150 µg/ml.) being most with 130 and 150 µg/ml. concentrations where there was complete cell death in all cultures of all donors starting from 48 hours of incubation onwards. At the concentration of 110 µg/ml., the quantitation estimates showed still viable cells up to 96 hours of incubation. The population variances of these cultures (σ^2) were estimated by 20.238, 28.572, 82.077 and 61.905 in the 70 µg/ml. concentration; 23.833, 28.572, 20.238 and 16.667 in the 90 µg/ml. concentration; 41.667, 39.287, 25.00 and 33.334 in the 110 µg/ml. concentration and 3.572,

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	120	160	205	240	622
Model A	70	200	100	110	120	160	390
	90	200	75	80	80	70	708
	110	200	35	30	20	10	16
	130	200	0	0	0	0	0
Model B	70	200	105	120	145	180	403
	90	200	85	95	90	85	716
	110	200	45	30	25	15	12
	130	200	0	0	0	0	0
Model C	70	200	120	140	170	190	445
	90	200	95	95	90	80	711
	110	200	55	40	30	20	9
	130	200	0	0	0	0	0
Model D	70	200	120	145	170	190	388
	90	200	105	95	95	90	600
	110	200	55	40	35	20	5
	130	200	0	0	0	0	0

Table 10 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 9 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	115	150	190	230	593
Model A	70	200	95	105	115	160	381
	90	200	65	80	85	70	701
	110	200	25	15	10	0	0
	130	200	0	0	0	0	0
Model B	70	200	105	120	135	185	407
	90	200	70	80	80	75	711
	110	200	35	20	10	5	10
	130	200	0	0	0	0	0
Model C	70	200	115	135	155	190	449
	90	200	80	90	95	75	713
	110	200	40	25	20	10	7
	130	200	0	0	0	0	0
Model D	70	200	120	135	160	195	383
	90	200	95	100	95	90	603
	110	200	45	30	20	10	5
	130	200	0	0	0	0	0

Table 11 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 10 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	125	160	210	260	630
Model A	70	200	90	105	115	150	367
	90	200	70	80	85	75	713
	110	200	30	20	15	5	11
	130	200	0	0	0	0	0
Model B	70	200	100	110	130	165	392
	90	200	85	90	95	85	725
	110	200	40	25	15	10	9
	130	200	10	5	0	0	0
Model C	70	200	115	125	150	180	436
	90	200	100	105	100	90	719
	110	200	45	30	20	10	6
	130	200	15	10	0	0	0
Model D	70	200	120	140	160	195	364
	90	200	100	110	110	105	599
	110	200	45	35	25	15	3
	130	200	15	10	5	0	0

Table 12 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No.11 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	115	145	195	235	601
Model A	70	200	95	110	120	165	385
	90	200	75	85	90	75	719
	110	200	35	20	10	0	0
	130	200	0	0	0	0	0
Model B	70	200	105	115	135	175	400
	90	200	85	95	90	75	729
	110	200	40	25	10	5	7
	130	200	0	0	0	0	0
Model C	70	200	105	125	150	185	449
	90	200	100	105	100	90	720
	110	200	45	25	15	10	5
	130	200	5	0	0	0	0
Model D	70	200	120	135	160	195	389
	90	200	100	105	105	100	611
	110	200	45	30	20	10	2
	130	200	10	5	0	0	0

Table 13 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 12 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	120	150	195	240	598
Model A	70	200	90	105	120	155	364
	90	200	70	85	80	80	732
	110	200	40	30	20	5	10
	130	200	0	0	0	0	0
Model B	70	200	100	115	135	160	386
	90	200	80	90	90	75	741
	110	200	45	30	20	10	8
	130	200	5	0	0	0	0
Model C	70	200	110	130	165	190	421
	90	200	95	100	100	90	732
	110	200	50	35	20	10	7
	130	200	15	10	0	0	0
Model D	70	200	115	130	170	195	369
	90	200	95	105	100	95	620
	110	200	50	35	25	15	4
	130	200	15	10	5	0	0

Table 14 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No.13 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	115	145	195	235	596
Model A	70	200	100	120	140	175	398
	90	200	80	95	90	80	726
	110	200	45	30	20	15	19
	130	200	5	0	0	0	0
Model B	70	200	110	125	140	180	404
	90	200	90	100	100	90	738
	110	200	55	40	25	15	13
	130	200	10	5	0	0	0
Model C	70	200	115	135	150	190	451
	90	200	105	115	115	100	729
	110	200	60	40	30	20	10
	130	200	15	10	0	0	0
Model D	70	200	120	150	180	200	390
	90	200	105	115	105	95	603
	110	200	60	45	30	25	6
	130	200	15	10	5	5	2

Table 15 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No.14 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control * = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	120	155	200	250	607
Model A	70	200	90	110	130	160	389
	90	200	70	85	80	75	737
	110	200	35	20	10	0	0
	130	200	0	0	0	0	0
Model B	70	200	100	120	145	175	399
	90	200	80	85	85	75	749
	110	200	40	25	10	10	9
	130	200	0	0	0	0	0
Model C	70	200	100	130	160	185	441
	90	200	95	90	85	75	746
	110	200	45	25	10	5	5
	130	200	0	0	0	0	0
Model D	70	200	105	130	170	195	395
	90	200	95	90	90	80	609
	110	200	55	30	15	5	2
	130	200	0	0	0	0	0

Table 16 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in donor No. 15 . Blast cells were scored out of 1000 counted lymphocytes (Experiment I).
Control * = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	118.6	152.0	198.6	241.4	606.7
Model A	70	200	94.3	109.3	122.9	160.7	382.0
	90	200	72.0	84.3	84.3	75.0	719.4
	110	200	35.0	23.6	15.0	5.0	8.0
	130	200	0.7	0.0	0.0	0.0	0.0
Model B	70	200	103.6	117.9	137.9	174.3	398.7
	90	200	82.0	90.7	90.0	80.0	729.9
	110	200	42.9	27.9	16.4	10.0	9.7
	130	200	3.6	1.4	0.0	0.0	0.0
Model C	70	200	111.4	131.4	157.0	187.0	441.7
	90	200	95.7	100.0	97.9	85.7	724.3
	110	200	48.6	31.4	20.7	12.0	7.0
	130	200	7.0	4.3	0.0	0.0	0.0
Model D	70	200	117.0	137.9	167.0	195.0	382.6
	90	200	99.3	102.9	100.0	93.6	606.4
	110	200	50.7	35.0	24.3	14.3	3.9
	130	200	7.9	5.0	2.0	0.7	0.3

Table 17 :- Showing the mean values of the cell kinetics and the transformation score in different culture models at different calcium ion concentrations in the female group (Experiment I). Control * = 50 µg/ml.

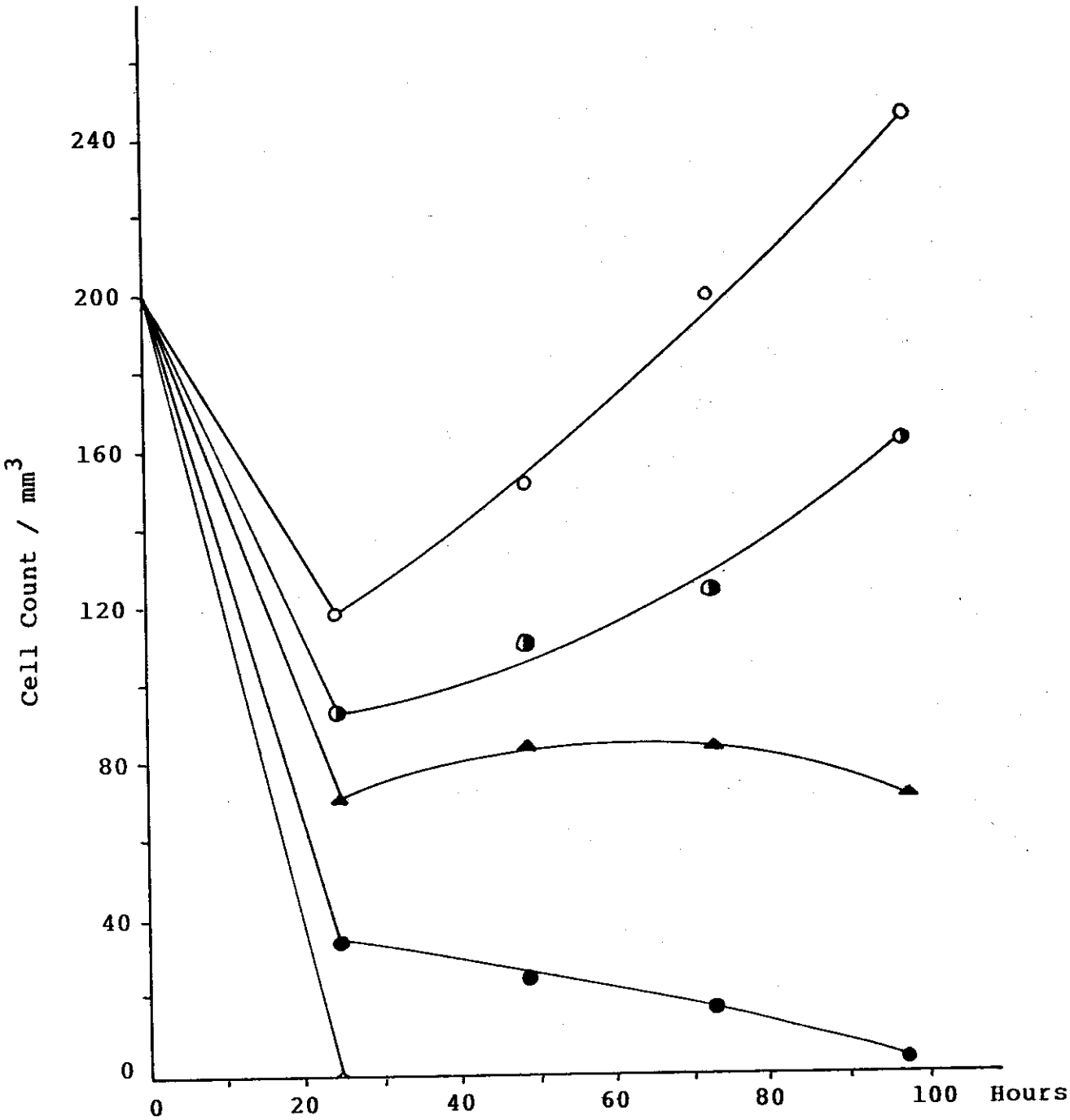

Fig.5:- Comparing the mean values of cell kinetics in the control and experimental cultures of the female donors in model A (Experiment I).O——O, control cultures; ◑——◑, 70 µg; ▲——▲,90 ug; ●——●, 110 µg and △——△ , 130 µg Ca^{++}/ml.

zero, zero and zero in the 130 µg/ml. concentration; all after 24, 48, 72 and 96 hours of incubation respectively.

Model B.

The quantitation estimates of the individual donors were tabulated in tables 10-16 and the mean values in table 17. A comparative plot was drawn to show the mean values of cell kinetics in the control and experimental cultures of model B after different hours of incubation (fig.6).

In this experimental model, there was a clear inverse relationship between the cell survival and the level of Ca^{++} concentration. After 24 hours of incubation, there was a sharp decline of the cell density with all tested concentrations (70-150 µg/ml.) and the quantitation estimates were all lower than the control value. At the concentration of 130 µg Ca^{++} per ml. of culture, there was complete cell death in the cultures of 4 donors (57%). In contrast, this zero value was exhibited in 100% of cultures subjected to the 150 µg/ml. concentration.

Prolongation of the culture period showed a steady recovery of the cellularity with the 70 µg/ml. concentration. However, at the 90 µg/ml. concentration, the count was nearly stable and the curve showed a blunt peak between 48 and 72 hour-incubation periods. With higher concentrations (110 and 130 µg/ml.), the sudden decline noticed at 24 hours of incubation was persistent and even progressive reaching its maximum at the 96 hour-incubation period. At the 130 µg/ml. concentration, complete cell death was observed in 5 cases (71%) as early as 48 hours of incubation and in all cases (100%) at 72 hour-incubation period.

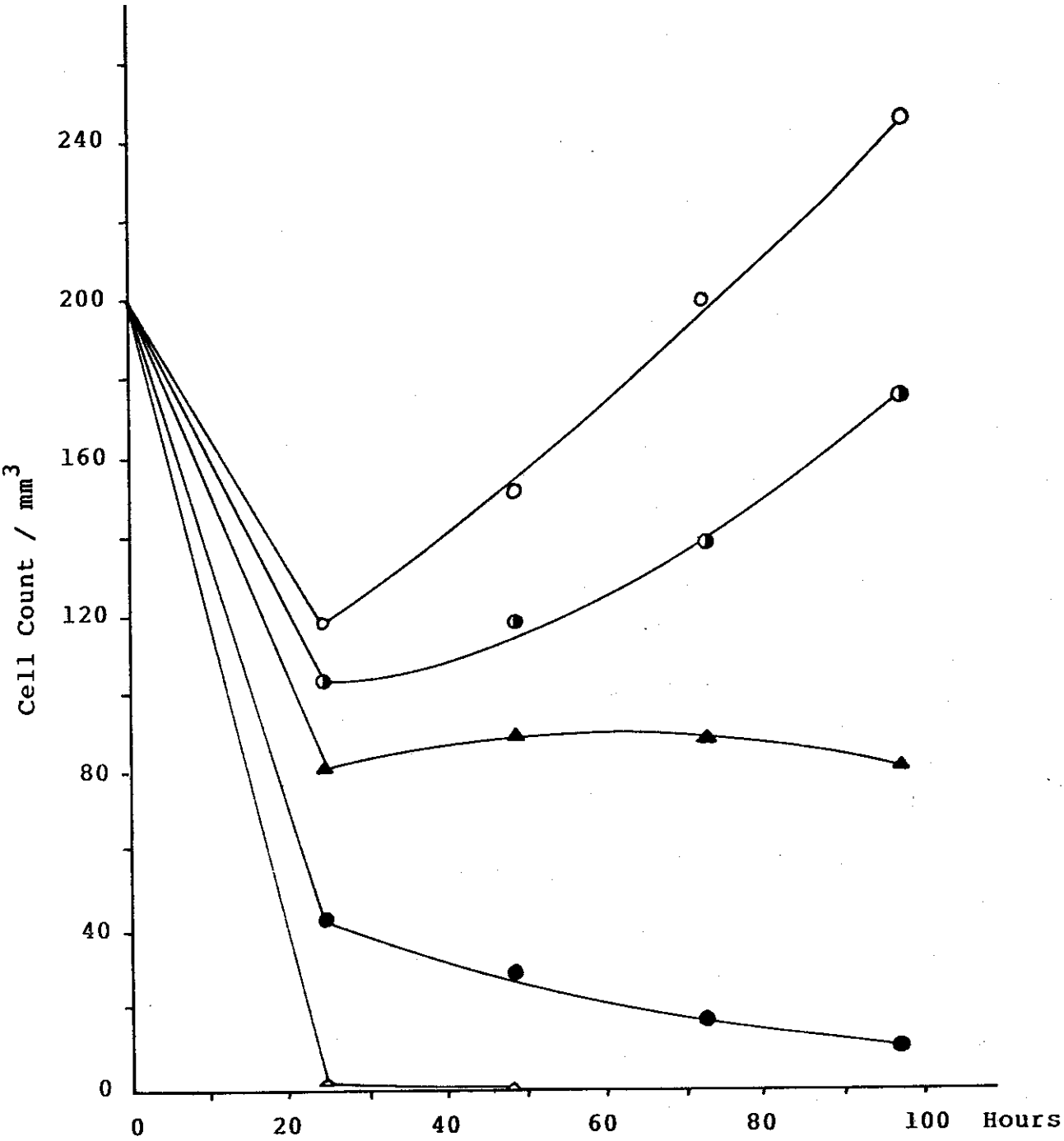

Fig.6:- Comparing the mean values of cell kinetics in the control and experimental cultures of the female donors in model B (Experiment I).O——O, control cultures; ◑——◑, 70 µg; ▲——▲ 90 µg; ●——●, 110 µg and △——△ , 130 µg Ca^{++}/ml.

The population variances (σ^2) of these cultures were calculated and found to be 14.287, 23.812, 32.145 and 78.572 in the 70 µg/ml. concentration; 40.5, 45.238, 41.667 and 41.667 in the 90 µg/ml. concentration; 40.478, 40.478, 45.953 and 16.667 in the 110 µg/ml. concentration and 22.62, 5.953, zero and zero in the 130 µg/ml. concentration; all after 24, 48, 72 and 96 hours of incubation respectively.

Model C.

The quantitation estimates of the cell kinetics were shown in tables 10-16 and the mean values in table 17. Fig.7 was plotted to compare the mean values of the cellular kinetics in the control and the experimental cultures of model C after different hour-incubation periods.

Herein, the same inverse relationship between the cell survival and the level of calcium ion concentration was observed in such the same way shown in model B. Nevertheless, the quantitation estimates were always higher than those obtained in model B and the lytic effect of high calcium ion concentrations (110 and 130 µg/ml.) was pronouncedly decreased.

The population variances (σ^2) of these cultures were estimated by 47.62, 30.953, 65.5 and 15.5 in the 70 µg/ml. concentration; 61.905, 83.334, 90.478 and 86.905 in the 90 µg/ml. concentration; 47.62, 47.62, 53.572 and 32.167 in the 110 µg/ml. concentration and 57.167, 28.572, zero and zero in the 130 µg/ml. concentration; all after 24, 48, 72 and 96 hours of incubation respectively.

Model D.

The quantitation estimates of the individual donors

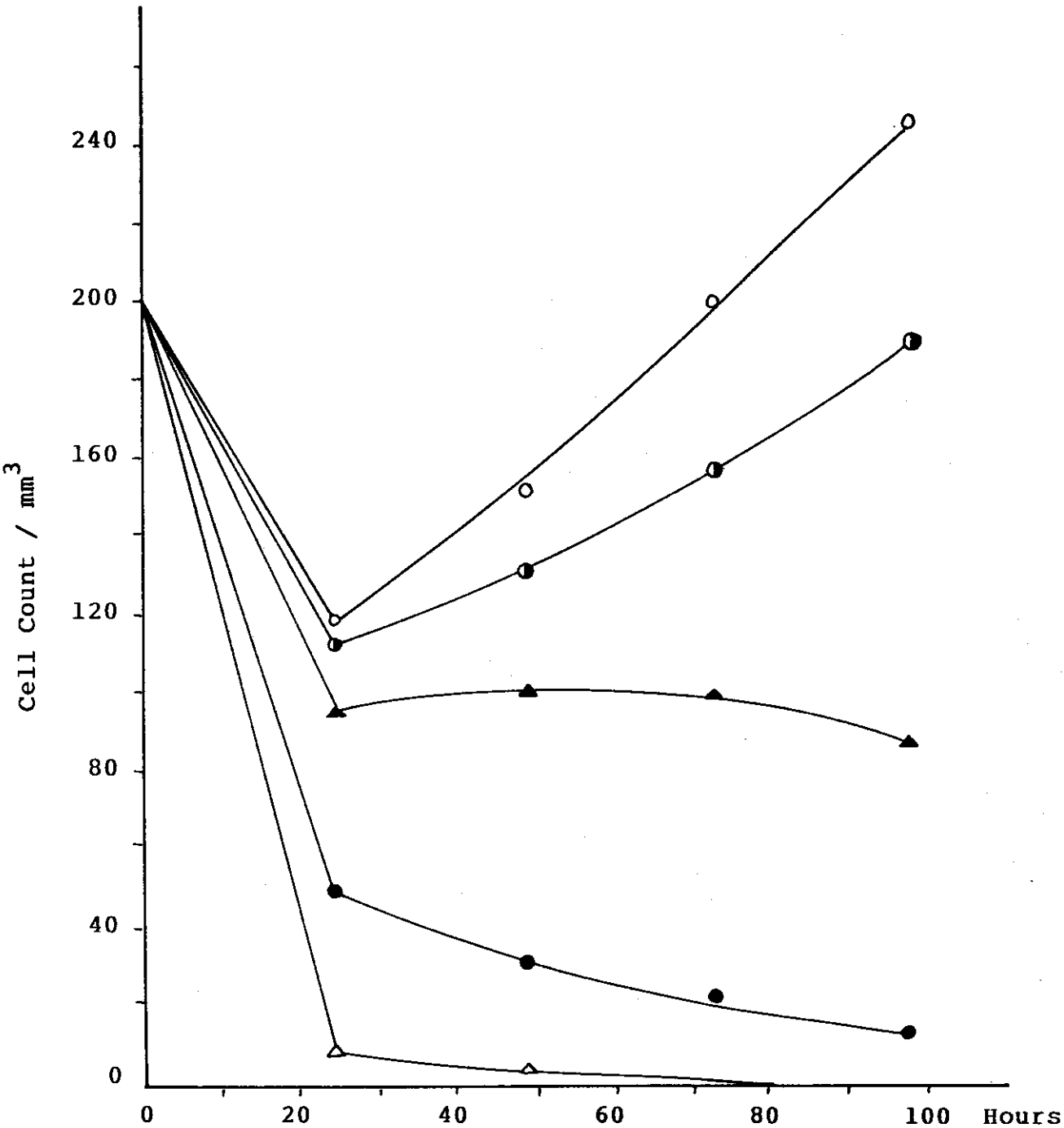

Fig.7:- Comparing the mean values of cell kinetics in the control and experimental cultures of the female donors in model C (Experiment I).O——O, control cultures; ◑——◑, 70 µg; ▲——▲ , 90 µg; ●——●, 110 µg and △——△ , 130 µg Ca^{++}/ml.

were shown in tables 10-16 and the mean values were presented in table 17. A comparative plot for the mean values of control cultures and those containing ascending concentrations of calcium ion (70-130 µg/ml.) in model D cultures was represented by fig.8.

In this experimental model, the 24 hours' decline of the cell density was observed, as well, in all experimental cultures. The maximum decline was observed with high calcium ion concentrations (110, 130 and 150 µg/ml.) whereas the 70 and 90 µg/ml. concentrations exhibited the same trend previously shown in the other experimental models. Nevertheless, the quantitation estimates of the 70 µg/ml. concentration were very close to the control values after 24 hour-incubation period and the curves were thence divergent from each other. Additionally, the lytic effect of the high Ca^{++} concentrations was not as dramatic as it had been in models A and B and still viable lymphocytes were observed up to 96 hours of incubation in cultures with 110 µg/ml. concentration in all donors. Regarding the effect of the 130 µg/ml. concentration, however, those viable lymphocytes were observed up to 72 hours of incubation in 3 cases (42.9%) and up to 96 hour-incubation period in only one case (14%). At the calcium ion concentration of 150 µg/ml., complete cell death was observed as early as 24 hours of incubation in all cultures of all donors.

The population variances (σ^2) of model D cultures were estimated by 32.167, 57.145, 57.167 and 8.333 in the 70 µg/ml. concentration; 20.238, 73.812, 50.00 and 64.287 in the 90 µg/ml. concentration; 36.905, 33.333, 45.238 and 45.238 in the 110 µg/ml. concentration and 57.145, 25.00, 7.167 and 3.572 in the 130 µg/ml. concentration; all after 24, 48, 72 and 96 hours of

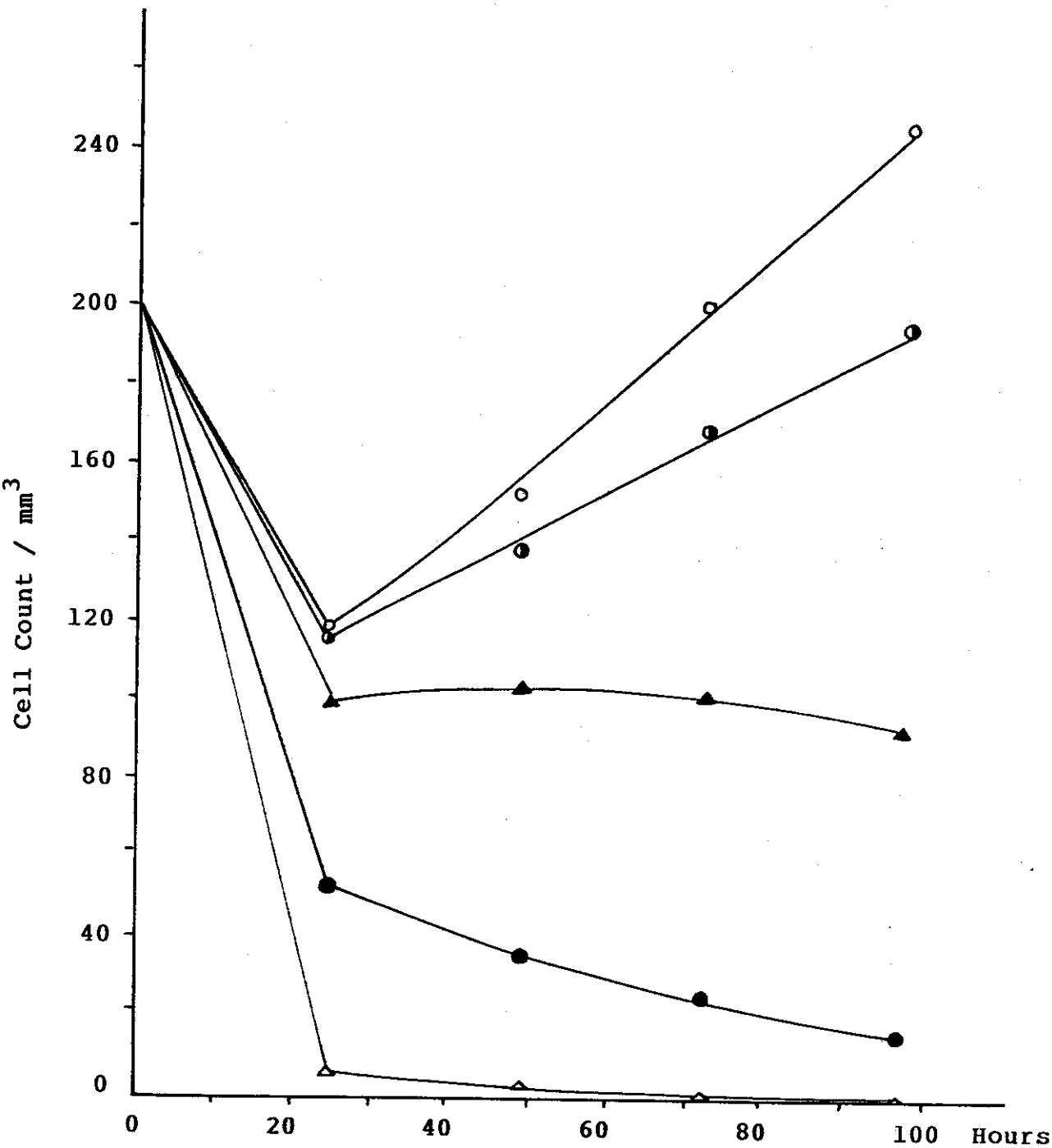

Fig.8:- Comparing the mean values of cell kinetics in the control and experimental cultures of the female donors in model D (Experiment I).O——O, control cultures; ◐——◐, 70 µg; ▲——▲, 90 ug; ●——●, 110 µg and △——△, 130 µg Ca^{++}/ml.

incubation respectively.

INDEX OF RESPONSE.

In this part of the present investigation, the capacity of blood lymphocytes to transform into blast cells under the influence of PHA-M was used to test the effect of calcium ion concentration on the process of mitogenesis and its relation to the turnover rate. The index of response was estimated by the percentage of transformed cells in 1000 smear-counted lymphocytes.

Group 1 (Male Donors):-

The quantitation estimates of the transformation score in the control as well as in the experimental cultures of the male group of donors were presented in tables 1-8 and the mean values in table 9.

In this group of donors, a clear variation of the transformation score was exhibited with different calcium ion concentrations. The highest scores were obtained with the 90 µg/ml. concentration in all experimental models and the mean values were always exceeding the control value. However, with the other tested calcium ion concentrations (70, 110 and 130 µg/ml.), the values were lower than those of the control smears being lowest with the 130 µg/ml. concentration (fig.9). As long as there were still viable lymphocytes, the transformed cell form could be found in the smears even in the deteriorating cultures subjected to the 110 and 130 µg/ml. concentrations (tables 1-9). In the smears prepared from control cultures as well as those obtained from the 70 and 90 µg/ml. cultures, a direct proportion between the transformation score and the cell density at 96 hour-incubation period was elicited. On the contrary, this relation was surprisingly reversed in the

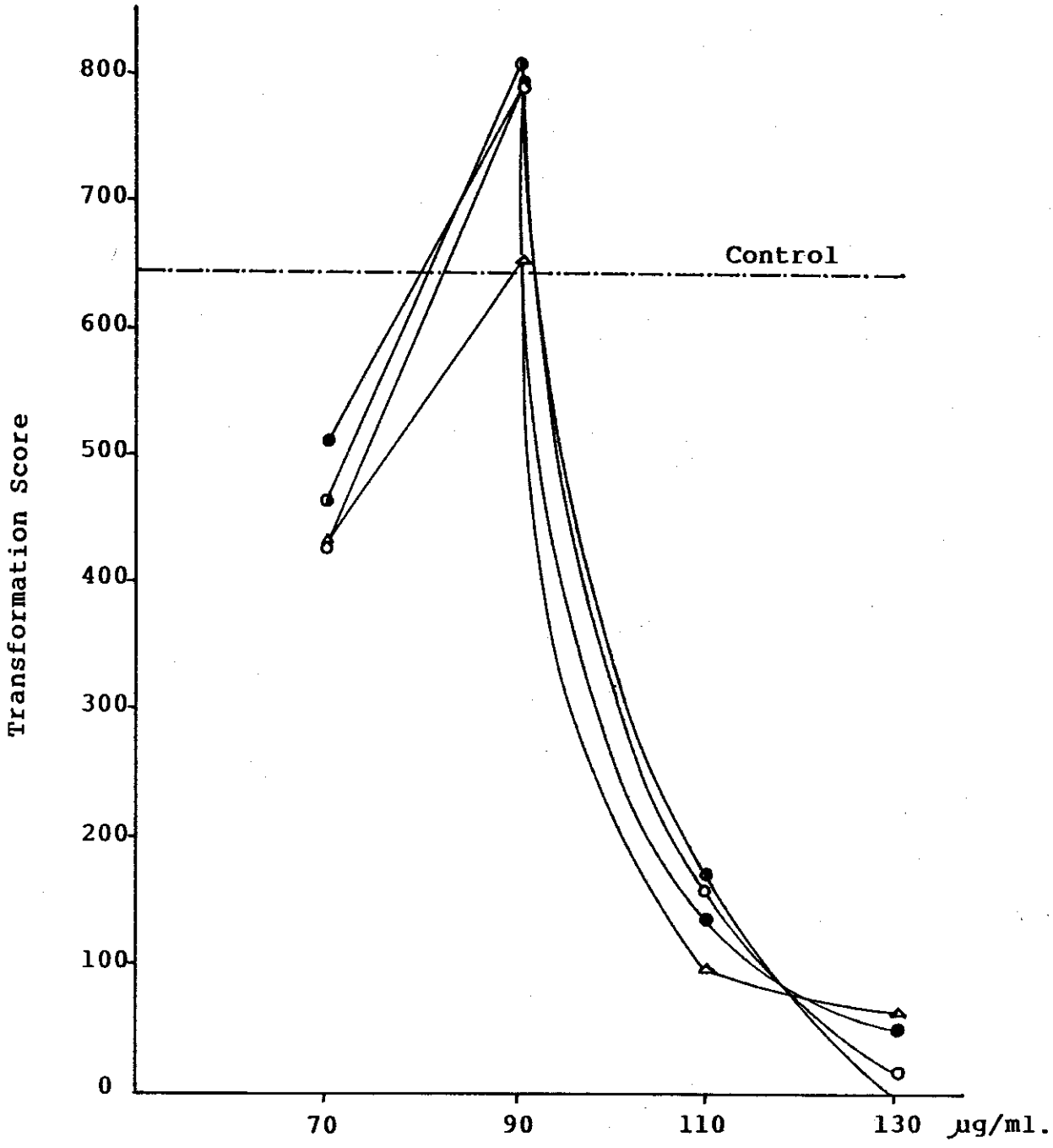

Fig. 9:- Showing the mean values of the transformation score in different experimental models at different calcium ion concentrations in the male group (Experiment I).
O———O, model A; ◐———◐, model B; ●——●, model C and △———△, model D.

deteriorating cultures subjected to the 110 µg/ml. concentration in all experimental models.

The population variances (σ^2) of these smears were calculated and found to be 18.439 in the control cultures and 3.314, 0.747, 1.483 and 2.023 in the 70 µg/ml. cultures; 0.396, 0.733, 0.033 and 0.229 in the 90 µg/ml. cultures; 1.024, 0.081, 0.18 and 0.157 in the 110 µg/ml. cultures and 0.405, zero, 0.193 and 0.282 in the 130 µg/ml. cultures; all in models A, B, C and D respectively.

Group 2 (Female Donors):-

The quantitation estimates of the transformation score in the control as well as in the experimental cultures of the female group of donors were presented in tables 10-16 and the mean values in table 17.

Herein,the transformation score was usually lower than its corresponding value in group 1 in the control as well as the other experimental models. However, the same configuration of the relationship between the index of response and the different calcium ion concentrations in different models was preserved (fig.10). The main difference was observed with the 130 µg/ml. concentration at which the lytic effect on the lymphocyte cell survival was pronounced and consequently no viable lymphocytes were available -after 96 hours of incubation- to prepare the smears.

The population variances (σ^2) of these smears were calculated and found to be 1.98 in the control cultures and 1.447, 0.54, 1.11 and 1.352 in the 70 µg/ml. concentration; 1.705, 1.882, 1.507 and 0.555 in the 90 µg/ml. concentration; 0.65, 0.046, 0.037 and 0.025 in the 110 µg/ml. concentration and zero, zero, zero and 0.006 in the 130 µg/ml. concentration; all in models

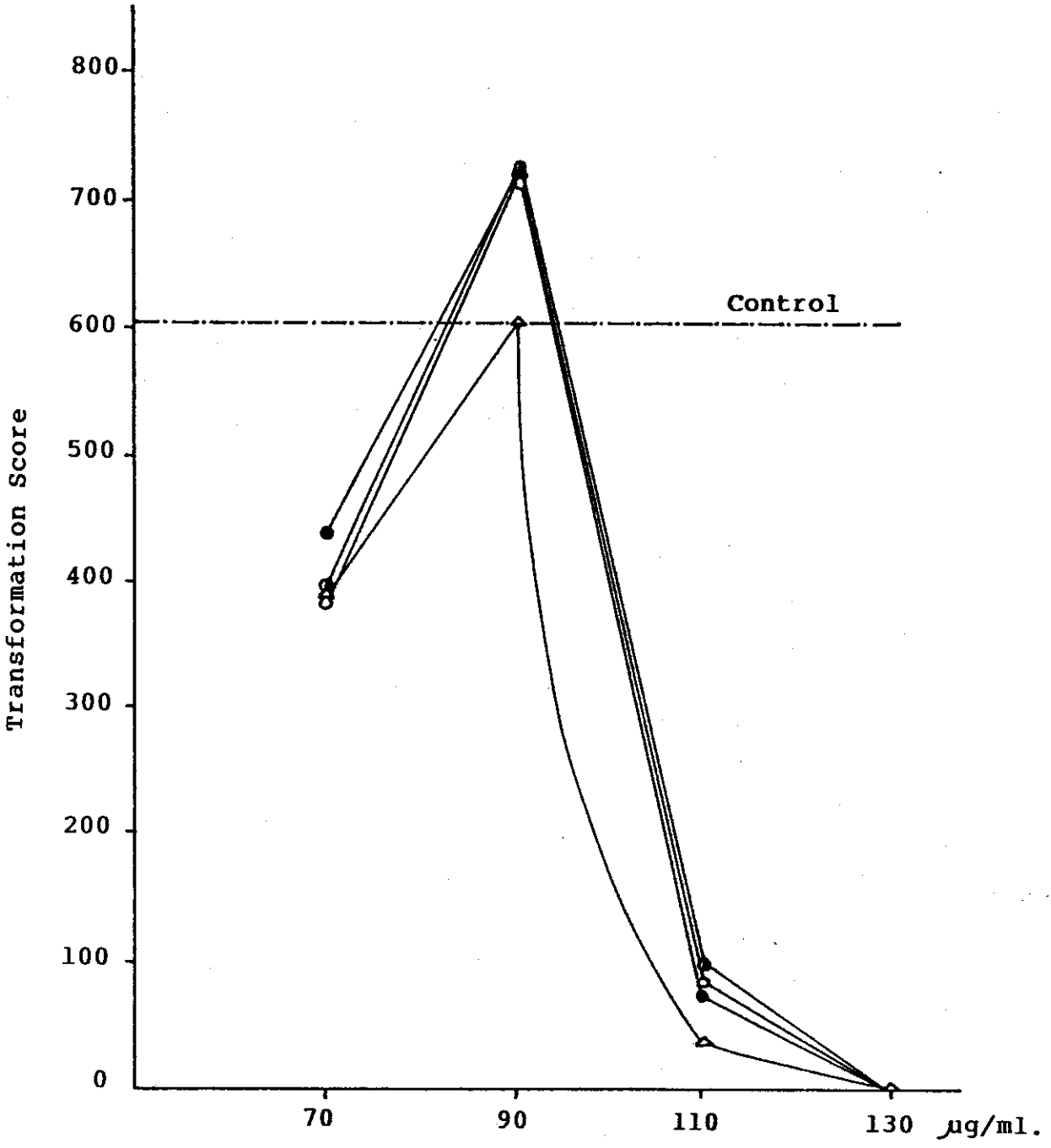

Fig. 10:- Showing the mean values of the transformation score in different experimental models at different calcium ion concentrations in the female group (Experiment I). O——O, model A; ◐——◐, model B; ●——●, model C and △——△, model D.

A, B, C and D respectively.

CHROMOSOME ANALYSIS.

This part of the study was performed on 50 metaphase cells in 10 randomly chosen slides from each culture tube. The normal karyotypes as well as the abnormals were counted and the differential percentages of the normal metaphases and those containing the different types of chromosome anomalies -met with- were calculated and presented in tables 18-25 (male donors) and in tables 27-33 (female donors). The mean values were represented by tables 26 and 34 in the male and female donors respectively.

Normal Metaphases.

The maximum yield of normal metaphases was observed in the control cultures as well as in the 70 µg/ml. concentration in all culture models in both the male and the female donors. In addition, the 130 µg/ml. concentration showed a very high yield of such karyotypes in model C of the male donors (table 26). Fig.11 and 12 were encountered to show the normal metaphase of a male cell and its chromosomal karyotyping respectively and figs.13 and 14 showed a normal female metaphase and its karyotyping respectively.

The population variances (σ^2) of these metaphase yields in the male group were found to be 2.211 in the control and 5.640, 11.429, 3.143 and 3.929 in the 70 µg/ml. concentration; 5.143, 4.497, 3.929 and 16.429 in the 90 µg/ml. concentration and 3.200, 2.286, 16.497 and 6.783 in the 110 µg/ml. concentration; all in models A, B, C and D respectively. Concerning the 130 µg/ml. concentration, no viable cells were available to prepare metaphase spreads in models A and B. However, the population variances (σ^2) of normal

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		98	2	0	0	0	0	0
Model A	70	96	2	2	0	0	0	0
	90	90	4	2	2	2	0	0
	110	86	2	8	2	2	0	0
	130	0	0	0	0	0	0	0
Model B	70	92	4	4	0	0	0	0
	90	88	4	2	0	2	4	0
	110	88	2	6	2	2	0	0
	130	0	0	0	0	0	0	0
Model C	70	94	2	4	0	0	0	0
	90	90	4	2	0	0	0	4
	110	88	0	8	2	2	0	0
	130	0	0	0	0	0	0	0
Model D	70	96	2	2	0	0	0	0
	90	82	2	6	0	0	10	0
	110	88	0	4	4	4	0	0
	130	0	0	0	0	0	0	0

Table 18 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.1 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		98	0	2	0	0	0	0
Model A	70	92	2	4	2	0	0	0
	90	84	2	4	0	2	0	8
	110	0	0	0	0	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	94	2	2	0	2	0	0
	90	90	2	2	4	0	0	2
	110	90	0	6	4	0	0	0
	130	0	0	0	0	0	0	0
Model C	70	96	0	2	0	2	0	0
	90	88	2	4	0	0	4	2
	110	94	0	6	0	0	0	0
	130	0	0	0	0	0	0	0
Model D	70	96	0	0	0	4	0	0
	90	74	0	4	4	2	8	8
	110	92	0	2	0	6	0	0
	130	84	2	8	4	2	0	0

Table 19 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.2 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control * = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		98	2	0	0	0	0	0
Model A	70	98	0	2	0	0	0	0
	90	88	2	2	0	4	4	0
	110	86	2	8	2	2	0	0
	130	0	0	0	0	0	0	0
Model B	70	98	2	0	0	0	0	0
	90	86	0	2	2	4	6	0
	110	90	0	4	2	4	0	0
	130	0	0	0	0	0	0	0
Model C	70	94	2	4	0	0	0	0
	90	92	0	4	2	0	2	0
	110	94	0	6	0	0	0	0
	130	94	0	2	2	2	0	0
Model D	70	92	2	4	2	0	0	0
	90	76	4	0	2	4	4	10
	110	88	0	8	4	0	0	0
	130	92	0	6	2	0	0	0

Table 20 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No. 3 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control * = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		96	4	0	0	0	0	0
Model A	70	94	2	4	0	0	0	0
	90	86	2	4	4	0	0	4
	110	88	0	4	4	4	0	0
	130	0	0	0	0	0	0	0
Model B	70	92	4	4	0	0	0	0
	90	90	2	4	0	0	0	4
	110	92	2	2	4	0	0	0
	130	0	0	0	0	0	0	0
Model C	70	98	2	0	0	0	0	0
	90	94	2	2	0	2	0	0
	110	84	0	8	4	4	0	0
	130	0	0	0	0	0	0	0
Model D	70	98	2	0	0	0	0	0
	90	84	2	4	0	2	8	0
	110	88	0	8	4	0	0	0
	130	0	0	0	0	0	0	0

Table 21 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.4 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control * = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		94	2	4	0	0	0	0
Model A	70	96	0	0	0	4	0	0
	90	86	0	0	0	4	10	0
	110	0	0	0	0	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	100	0	0	0	0	0	0
	90	92	2	4	0	2	0	0
	110	92	0	4	2	2	0	0
	130	0	0	0	0	0	0	0
Model C	70	98	0	2	0	0	0	0
	90	94	0	2	0	4	0	0
	110	88	2	8	2	0	0	0
	130	96	0	0	2	2	0	0
Model D	70	96	2	2	0	0	0	0
	90	82	2	6	0	0	10	0
	110	90	0	4	6	0	0	0
	130	90	2	8	0	0	0	0

Table 22 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.5 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		96	2	2	0	0	0	0
Model A	70	96	2	0	0	2	0	0
	90	90	0	2	4	0	4	0
	110	88	0	8	4	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	94	2	2	2	0	0	0
	90	88	2	2	0	4	0	4
	110	90	0	4	2	4	0	0
	130	0	0	0	0	0	0	0
Model C	70	98	2	0	0	0	0	0
	90	92	2	4	0	0	2	0
	110	96	0	4	0	0	0	0
	130	100	0	0	0	0	0	0
Model D	70	98	0	0	0	2	0	0
	90	86	0	0	2	4	0	8
	110	88	0	8	0	4	0	0
	130	0	0	0	0	0	0	0

Table 23 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.6 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).

Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		96	0	4	0	0	0	0
Model A	70	92	2	2	4	0	0	0
	90	88	2	4	2	0	0	4
	110	90	2	4	2	2	0	0
	130	90	2	8	0	0	0	0
Model B	70	100	0	0	0	0	0	0
	90	88	0	4	2	0	6	0
	110	90	0	6	2	2	0	0
	130	0	0	0	0	0	0	0
Model C	70	96	2	2	0	0	0	0
	90	92	2	2	4	0	0	0
	110	88	0	8	4	0	0	0
	130	98	2	0	0	0	0	0
Model D	70	94	2	4	0	0	0	0
	90	82	2	6	0	0	0	10
	110	92	0	2	6	0	0	0
	130	88	2	8	2	0	0	0

Table 24 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No. 7 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).

Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		98	2	0	0	0	0	0
Model A	70	98	2	0	0	0	0	0
	90	90	2	4	0	0	4	0
	110	90	0	8	2	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	98	2	0	0	0	0	0
	90	92	2	2	4	0	0	0
	110	88	0	6	4	2	0	0
	130	0	0	0	0	0	0	0
Model C	70	98	2	0	0	0	0	0
	90	92	2	2	0	0	0	4
	110	94	0	6	0	0	0	0
	130	98	0	0	2	0	0	0
Model D	70	96	2	2	0	0	0	0
	90	78	2	4	0	0	8	8
	110	84	0	8	4	4	0	0
	130	90	0	6	0	4	0	0

Table 25 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No. 8 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		96.75	1.75	1.50	0	0	0	0
Model A	70	95.25	1.50	1.75	0.75	0.75	0	0
	90	88.00	1.75	2.75	1.50	1.50	2.75	2.00
	110	88.00	1.00	6.66	2.66	1.66	0	0
	130	0	0	0	0	0	0	0
Model B	70	96.00	2.00	1.50	0.25	0.25	0	0
	90	89.25	1.75	2.75	1.50	1.50	2.00	1.25
	110	90.00	0.50	4.75	2.75	2.00	0	0
	130	0	0	0	0	0	0	0
Model C	70	96.50	1.50	1.75	0	0.25	0	0
	90	91.75	1.75	2.75	0.75	0.75	1.00	1.25
	110	90.75	0.25	6.75	1.50	0.75	0	0
	130	97.20	0.40	0.40	1.20	0.80	0	0
Model D	70	95.75	1.50	1.75	0.25	0.75	0	0
	90	80.50	1.75	3.75	1.00	1.50	6.00	5.50
	110	88.75	0	5.50	3.50	2.25	0	0
	130	88.80	1.20	7.20	1.60	1.20	0	0

Table 26 :- Showing the mean values of the differential percentages of the metaphase karyotypes in the control and the experimental cultures of the male group. N (normal), H (hypodiploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		98	0	2	0	0	0	0
Model A	70	96	2	2	0	0	0	0
	90	78	2	2	4	2	6	6
	110	82	2	10	6	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	96	2	2	0	0	0	0
	90	84	0	2	4	4	6	0
	110	84	0	8	4	4	0	0
	130	0	0	0	0	0	0	0
Model C	70	96	2	2	0	0	0	0
	90	88	2	4	0	2	4	0
	110	82	0	10	4	4	0	0
	130	0	0	0	0	0	0	0
Model D	70	94	2	2	2	0	0	0
	90	72	4	4	0	6	8	6
	110	82	0	10	8	0	0	0
	130	0	0	0	0	0	0	0

Table 27 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.9 . N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		94	2	4	0	0	0	0
Model A	70	94	0	4	0	2	0	0
	90	78	2	4	0	4	8	4
	110	0	0	0	0	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	94	0	0	2	4	0	0
	90	82	2	4	2	0	4	6
	110	88	0	8	4	0	0	0
	130	0	0	0	0	0	0	0
Model C	70	96	2	0	0	2	0	0
	90	88	2	4	2	0	0	4
	110	86	2	6	6	0	0	0
	130	0	0	0	0	0	0	0
Model D	70	94	2	4	0	0	0	0
	90	74	2	6	4	0	6	8
	110	84	2	8	0	6	0	0
	130	0	0	0	0	0	0	0

Table 28 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.10. N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control * = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		98	2	0	0	0	0	0
Model A	70	96	2	0	2	0	0	0
	90	84	4	2	2	2	0	6
	110	78	2	10	4	6	0	0
	130	0	0	0	0	0	0	0
Model B	70	94	2	2	0	2	0	0
	90	84	4	4	0	4	0	4
	110	86	0	8	2	4	0	0
	130	0	0	0	0	0	0	0
Model C	70	96	0	2	0	2	0	0
	90	88	0	4	0	4	4	0
	110	84	0	10	0	6	0	0
	130	0	0	0	0	0	0	0
Model D	70	92	0	2	4	2	0	0
	90	74	2	4	2	4	8	6
	110	84	0	10	0	6	0	0
	130	0	0	0	0	0	0	0

Table 29 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.11. N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		94	4	2	0	0	0	0
Model A	70	92	2	2	0	4	0	0
	90	84	2	4	0	4	6	0
	110	0	0	0	0	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	92	4	4	0	0	0	0
	90	84	2	4	2	2	6	0
	110	90	0	6	4	0	0	0
	130	0	0	0	0	0	0	0
Model C	70	94	2	4	0	0	0	0
	90	84	2	2	4	0	2	6
	110	86	2	6	6	0	0	0
	130	0	0	0	0	0	0	0
Model D	70	96	0	0	0	4	0	0
	90	72	0	4	4	6	8	6
	110	84	0	10	0	6	0	0
	130	0	0	0	0	0	0	0

Table 30 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.12. N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).

Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		98	2	0	0	0	0	0
Model A	70	96	0	0	4	0	0	0
	90	84	2	2	4	0	8	0
	110	82	0	6	6	6	0	0
	130	0	0	0	0	0	0	0
Model B	70	94	2	0	4	0	0	0
	90	80	2	2	4	2	6	4
	110	88	2	4	6	0	0	0
	130	0	0	0	0	0	0	0
Model C	70	96	0	2	2	0	0	0
	90	92	2	4	2	0	0	0
	110	90	0	6	4	0	0	0
	130	0	0	0	0	0	0	0
Model D	70	94	2	0	2	2	0	0
	90	78	2	4	2	0	6	8
	110	84	0	8	8	0	0	0
	130	0	0	0	0	0	0	0

Table 31 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.13. N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		98	2	0	0	0	0	0
Model A	70	96	2	2	0	0	0	0
	90	86	0	4	0	4	0	6
	110	80	2	10	6	2	0	0
	130	0	0	0	0	0	0	0
Model B	70	94	2	4	0	0	0	0
	90	82	2	2	4	4	0	6
	110	86	0	6	4	4	0	0
	130	0	0	0	0	0	0	0
Model C	70	94	2	4	0	0	0	0
	90	92	4	2	0	2	0	0
	110	90	0	10	0	0	0	0
	130	0	0	0	0	0	0	0
Model D	70	94	2	4	0	0	0	0
	90	80	2	6	0	4	8	0
	110	82	2	4	8	4	0	0
	130	86	2	8	2	2	0	0

Table 32 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.14. N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		94	2	4	0	0	0	0
Model A	70	94	2	4	0	0	0	0
	90	80	2	4	2	0	8	4
	110	0	0	0	0	0	0	0
	130	0	0	0	0	0	0	0
Model B	70	96	4	0	0	0	0	0
	90	80	2	4	2	2	4	6
	110	80	0	8	6	6	0	0
	130	0	0	0	0	0	0	0
Model C	70	98	2	0	0	0	0	0
	90	84	2	4	2	2	0	6
	110	84	0	10	0	6	0	0
	130	0	0	0	0	0	0	0
Model D	70	92	2	2	0	4	0	0
	90	76	2	4	4	0	6	8
	110	86	0	8	6	0	0	0
	130	0	0	0	0	0	0	0

Table 33 :- Showing the differential percentages of 50 metaphase karyotypes in the control and the experimental cultures of donor No.15. N (normal), H (hypo-diploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control * = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		96.29	2.00	1.71	0	0	0	0
Model A	70	94.86	1.71	2.00	0.86	0.86	0	0
	90	82.00	2.00	3.14	1.71	2.29	5.14	3.71
	110	80.50	1.50	9.00	5.50	3.50	0	0
	130	0	0	0	0	0	0	0
Model B	70	94.29	2.29	1.71	0.86	0.86	0	0
	90	82.29	2.00	3.14	2.57	2.57	3.71	3.71
	110	86.00	0.29	6.86	4.29	2.57	0	0
	130	0	0	0	0	0	0	0
Model C	70	95.71	1.43	2.00	0.29	0.57	0	0
	90	88.00	2.00	3.43	1.43	1.43	1.43	2.29
	110	86.00	0.57	8.29	2.86	2.29	0	0
	130	0	0	0	0	0	0	0
Model D	70	93.71	1.43	2.00	1.14	1.71	0	0
	90	75.14	2.00	4.57	2.29	2.86	7.14	6.00
	110	83.71	0.57	8.29	4.29	3.14	0	0
	130	0	0	0	0	0	0	0

Table 34 :- Showing the mean values of the differential percentages of the metaphase karyotypes in the control and the experimental cultures of the female group. N (normal), H (hypodiploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploid) and E (endoreduplication).
Control * = 50 µg/ml.

Fig. 11:- Showing a photomicrograph of a normal metaphase spread prepared from a male control culture. Note the peripheral position of the Y chromosome (arrow). (Giemsa stain, x 1200).

Fig. 12:- Showing a karyotype of a normal male chromosome complement (46, XY).

Fig. 13:- Showing a photomicrograph of a normal metaphase spread prepared from a female control culture.
(Giemsa stain, X 1200).

Fig. 14:- Showing a karyotype of a normal female chromosome complement (46, XX).

metaphases were estimated by 5.200 and 9.200 in models C and D respectively.

In the female group, the corresponding values of the population variances (σ^2) were estimated by 3.931 in the control and 2.480, 1.905, 1.905 and 1.905 in the 70 µg/ml. concentration; 10.666, 3.238, 10.666 and 7.945 in the 90 µg/ml. concentration and 3.000, 10.666, 9.333 and 1.905 in the 110 µg/ml. concentration; all in models A, B, C and D respectively.

Abnormal Metaphases.

The main types of numerical chromosomal anomalies met with in this study were in the form of hypodiploid chromosome complements (figs.15 and 16), tetraploidy (fig.17) and endoreduplication (fig.18). The structural anomalies comprised breaks (figs.19 & 20), dicentric chromosome complements (fig.21) and ring chromosome content (fig.22).

The highest yields of the above mentioned abnormalities were observed in the 90 and the 110 µg/ml. concentrations in all experimental models in both group 1 (male donors) and group 2 (female donors). In addition, the 130 µg/ml. concentration of model D showed a high incidence of such abnormal karyotypes in the male group (table 26).

In the male group, the mean percentage of chromosomal anomalies ranged from 8.25 to 19.5% in the 90 µg/ml. concentration and from 9.25 to 12% in the 110 µg/ml. concentration in the different experimental models whereas a mean percentage of 3.25% was noted in the control cultures. In contrast, the female smears showed a range of 12 to 24.9% and 14 to 19.5% in the mean percentage of total chromosomal anomalies in the

Fig. 15:- Showing a photomicrograph of a metaphase spread with hypodiploid complement prepared from a male control culture.
(Giemsa stain, X 1200).

Fig. 16:- Showing a male karyotype with 45 chromosomes including monosomy of chromosome No.14 (45, XY, -14).

Fig. 17:- Showing a photomicrograph of a metaphase spread with a tetraploid chromosome complement prepared from a female 90 µg/ml. culture.
(Giemsa stain, X 1200).

Fig. 18:- Showing a photomicrograph of a metaphase spread with endoreduplication prepared from a female 90 µg/ml. culture.
(Giemsa stain, X 1200).

Fig. 19:- Showing a photomicrograph of a metaphase spread with a chromatid gap in a group-C chromosome (arrow) prepared from a male 110 µg/ml. culture.
(Giemsa stain, X 1200).

Fig. 20:- Showing a male karyotype with a gap in one of the long arms of chromosome No.12 (46, XY, 12q:).

Fig. 21:- Showing a photomicrograph of a metaphase spread with one dicentric chromosome and two acentric rings (arrows) prepared from a male 110 µg/ml. culture.
(Giemsa stain, X 1200).

Fig. 22:- Showing a photomicrograph of a metaphase spread with a ring chromosome and an acentric fragment (arrows) prepared from a female 110 µg/ml. culture.
(Giemsa stain, X 1200).

90 and the 110 µg/ml. concentrations respectively. The control cultures had a mean incidence percentage of 3.7%.

Of interest was the finding that chromosomal breaks constituted the majority of the total abnormalities at the 110 µg/ml. concentration in all experimental models in both groups of donors whereas tetraploidy and endoreduplication showed a tendency of high incidence in the 90 µg/ml. concentration in all experimental models (tables 26 and 34). The maximum incidence of chromosomal breaks was observed in the 130 µg/ml. concentration of model D in the male donors (6-8% of total cells examined).

The population variances (σ^2) of the total abnormal metaphases in the male group were estimated by 2.211 in the control and 4.560, 11.429, 3.143 and 3.929 in the 70 µg/ml. concentration; 5.143, 4.497, 3.929 and 16.857 in the 90 µg/ml. concentration and 3.200, 3.200, 18.211 and 6.783 in the 110 µg/ml. concentration; all in models A, B, C and D respectively. The 130 µg/ml. concentration had a population variance (σ^2) of 5.200 in model C and 9.200 in model D.

In the female group, the corresponding values of the population variances (σ^2) were found to be 4.572 in the control and 2.478, 1.905, 1.905 and 1.905 in the 70 µg/ml. concentration; 10.666, 3.238, 10.666 and 9.145 in the 90 µg/ml. concentration and 3.666, 10.666, 9.333 and 1.905 in the 110 µg/ml. concentration; all in models A, B, C and D respectively.

EXPERIMENT II.

The primary objective of this experiment was to formulate some sort of comparison between the effect of ascending concentrations of calcium ion on the cell kinetics, the mitogenesis and the chromosomal structure of "in vitro" grown lymphocytes activated by the plant lectin phytohaemagglutinin (Experiment I), with their effect on lymphocytes activated in response to histocompatibility antigen. Five mixed-lymphocyte culture series were set up in this experiment namely; two culture series from 4 male donors (male-versus-male), two from 4 female donors (female-versus-female) and one culture series utilizing the blood samples of one male and one female (male-versus-female).

CELL KINETICS.

In this part of the study, the cell density was estimated 24 hourly begining by the time of inoculation (Time 0) and ending at 96 hours of incubation. Out of the 5 culture series utilized, two were deteriorating viz; one from the male donors (table 35) and one from the female donors (table 38). In these deteriorating cultures, the 24 hours' decline of the cell density was followed by persistent and progressive loss of viable cells as deduced from the estimates of cell density after 48, 72 and 96 hours of incubation.

Interestingly, the results obtained from the remaining male culture and the remaining female one were highly analogous to those achieved in their corresponding cultures of experiment I (tables 36 and 37). Surprisingly, the third successful culture seemingly had a midway-behaviour between the cell kinetics of the male and the female groups of experiment I even in the control cultures (table 39). Collectively, there

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	100	70	40	15	0
Model A	70	200	110	70	45	20	0
	90	200	80	55	30	5	0
	110	200	40	25	10	0	0
	130	200	25	15	0	0	0
Model B	70	200	105	80	55	20	0
	90	200	90	55	35	10	0
	110	200	45	30	10	0	0
	130	200	25	15	5	0	0
Model C	70	200	110	80	60	25	0
	90	200	85	60	35	15	0
	110	200	40	30	10	0	0
	130	200	30	15	10	0	0
Model D	70	200	120	90	70	35	0
	90	200	115	70	40	20	0
	110	200	60	40	25	10	0
	130	200	40	0	0	0	0

Table 35 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in culture No.1. Blast cells were scored out of 1000 counted lymphocytes (Experiment II). Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	95	105	115	145	676
Model A	70	200	115	150	190	225	429
	90	200	80	90	95	85	792
	110	200	40	20	10	5	19
	130	200	20	10	5	0	0
Model B	70	200	95	115	130	160	442
	90	200	90	95	100	90	799
	110	200	50	35	20	10	11
	130	200	30	15	10	0	0
Model C	70	200	105	130	155	210	490
	90	200	100	100	105	95	782
	110	200	65	45	30	15	9
	130	200	40	25	10	5	20
Model D	70	200	120	150	195	235	435
	90	200	105	115	115	100	718
	110	200	70	55	30	20	5
	130	200	45	30	15	10	12

Table 36 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in culture No.2. Blast cells were scored out of 1000 counted lymphocytes (Experiment II). Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control *		200	120	150	200	235	613
Model A	70	200	100	135	175	195	400
	90	200	80	85	90	75	730
	110	200	30	20	10	5	21
	130	200	15	10	0	0	0
Model B	70	200	90	110	130	150	411
	90	200	85	90	90	80	737
	110	200	40	25	15	10	17
	130	200	20	10	5	0	0
Model C	70	200	95	120	140	190	430
	90	200	90	95	95	90	729
	110	200	50	30	20	10	18
	130	200	25	10	5	0	0
Model D	70	200	105	135	180	200	398
	90	200	95	90	90	85	689
	110	200	65	40	30	15	12
	130	200	35	25	10	5	16

Table 37 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in culture No.3. Blast cells were scored out of 1000 counted lymphocytes (Experiment II). Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	95	60	30	10	0
Model A	70	200	100	70	40	20	0
	90	200	80	40	15	0	0
	110	200	5	0	0	0	0
	130	200	0	0	0	0	0
Model B	70	200	105	75	50	25	0
	90	200	80	40	15	5	0
	110	200	15	5	0	0	0
	130	200	0	0	0	0	0
Model C	70	200	110	75	55	35	0
	90	200	90	40	15	10	0
	110	200	15	10	5	0	0
	130	200	5	0	0	0	0
Model D	70	200	115	80	50	40	0
	90	200	90	60	45	20	0
	110	200	30	15	5	0	0
	130	200	10	5	0	0	0

Table 38 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in culture No.4. Blast cells were scored out of 1000 counted lymphocytes (Experiment II). Control* = 50 µg/ml.

Ca Ions in µg / ml.		Cell Count / mm^3 of Culture					Blast Cells
		Time 0	24 hr.	48 hr.	72 hr.	96 hr.	
Control*		200	90	100	115	140	640
Model A	70	200	105	140	180	205	410
	90	200	80	90	90	80	760
	110	200	30	20	10	5	19
	130	200	20	10	5	5	21
Model B	70	200	90	110	125	155	423
	90	200	80	90	95	85	771
	110	200	45	30	20	10	15
	130	200	30	15	10	5	18
Model C	70	200	100	120	150	195	452
	90	200	90	95	95	85	763
	110	200	55	40	25	15	11
	130	200	30	20	15	10	13
Model D	70	200	115	140	180	220	416
	90	200	95	105	100	90	709
	110	200	60	40	25	15	12
	130	200	40	25	10	5	18

Table 39 :- Showing the cell kinetics in different culture models at different calcium ion concentrations in culture No.5. Blast cells were scored out of 1000 counted lymphocytes (Experiment II).
Control* = 50 µg/ml.

was a sharp decline of the cell density after 24 hour-incubation period followed by a relapse thenceafter except in cultures subjected to the 110 and 130 µg/ml. concentrations which showed a progressive decline of the cell density. The 90 µg/ml. concentration showed the same stationary cell density with a slight blunt peak of cellularity between 48 and 72 hours of incubation. In contrast, in cultures subjected to the 70 µg/ml. concentration, the cell density was ever higher than the standard control cultures after the corresponding incubation periods in all experimental models.

INDEX OF RESPONSE.

In analogy to what had been described in experiment I, the maximum yield of blastoid cells was observed with the 90 µg/ml. concentration in the three successful cultures in all experimental models (table 36, male-versus-male; table 37, female-versus-female and table 39, male-versus-female). However, the two deteriorating cultures showed none of those transformed cells even in cultures still having viable lymphocytes at the time of smear preparation (table 35, male-versus-male and table 38, female-versus-female). In addition, an inverse relationship between the cell survival and the blast cell percentage was noticed in the 110 and 130 µg/ml. concentrations in all experimental models (tables 36, 37 and 39).

CHROMOSOME ANALYSIS.

In this part of the study, the differential percentage of the normal and abnormal karyotypes were calculated using 100 metaphase cells in each group of smears prepared from each culture tube. Tables 40 (male-versus-male), 41 (female-versus-female) and 42 (male-versus-female) were encountered to demonstrate that

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		97	2	1	0	0	0	0
Model A	70	95	2	1	1	1	0	0
	90	89	1	3	1	1	3	2
	110	88	1	7	2	2	0	0
	130	0	0	0	0	0	0	0
Model B	70	95	1	2	1	1	0	0
	90	88	2	3	1	1	3	2
	110	89	0	6	3	2	0	0
	130	0	0	0	0	0	0	0
Model C	70	98	1	1	0	0	0	0
	90	90	1	2	2	0	3	2
	110	89	0	8	2	1	0	0
	130	95	0	3	1	1	0	0
Model D	70	95	2	1	1	1	0	0
	90	82	2	3	2	2	5	4
	110	86	0	8	3	3	0	0
	130	83	1	10	2	4	0	0

Table 40 :- Showing the differential percentages of the karyotypes of 100 counted metaphases in culture series No.2 (Experiment II). N (normal), H (hypodiploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploidy) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control*		96	2	2	0	0	0	0
Model A	70	93	2	2	1	2	0	0
	90	86	1	3	2	1	4	3
	110	83	2	9	3	3	0	0
	130	0	0	0	0	0	0	0
Model B	70	95	2	2	1	0	0	0
	90	79	1	4	3	2	5	6
	110	84	1	8	4	3	0	0
	130	0	0	0	0	0	0	0
Model C	70	96	2	2	0	0	0	0
	90	83	2	4	2	1	4	4
	110	82	1	12	2	3	0	0
	130	0	0	0	0	0	0	0
Model D	70	93	1	2	2	2	0	0
	90	78	1	5	2	2	7	5
	110	78	1	14	3	4	0	0
	130	77	0	14	5	4	0	0

Table 41 :- Showing the differential percentages of the karyotypes of 100 counted metaphases in culture series No.3 (Experiment II). N (normal), H (hypodiploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploidy) and E (endoreduplication).
Control* = 50 µg/ml.

Ca Ions in µg/ml.		Differential % of 50 Metaphase Karyotypes						
		N	H	B	R	D	T	E
Control *		97	1	2	0	0	0	0
Model A	70	96	2	1	0	1	0	0
	90	89	1	2	1	1	3	3
	110	87	0	8	2	3	0	0
	130	85	1	9	3	2	0	0
Model B	70	96	1	1	1	1	0	0
	90	84	2	4	2	0	4	4
	110	87	1	6	3	3	0	0
	130	85	0	8	3	4	0	0
Model C	70	97	1	2	0	0	0	0
	90	90	0	3	1	1	3	2
	110	85	1	9	3	2	0	0
	130	79	0	12	5	4	0	0
Model D	70	94	1	2	2	1	0	0
	90	83	1	4	2	1	5	4
	110	81	2	11	3	3	0	0
	130	80	1	13	2	4	0	0

Table 42 :- Showing the differential percentages of the karyotypes of 100 counted metaphases in culture series No.5 (Experiment II). N (normal), H (hypodiploid), B (breaks), R (ring chromosome), D (dicentric chromosome), T (tetraploidy) and E (endoreduplication).
Control * = 50 µg/ml.

the maximum yield of abnormal metaphases was noticed with high calcium ion concentrations (90-130 µg/ml.) in all experimental models in all successful cultures. In analogy to the results of experiment I, the highest percentage of tetraploidy and endoreduplication was observed in the 90 µg/ml. concentration in all experimental models. In addition, breaks tended to predominate in the concentrations of 110 and 130 µg/ml. with a noticeable higher incidence in the latter concentration. Comparing the total abnormal metaphase percentages in pure male mixed-lymphocyte culture series (table 40) with those in pure female mixed-lymphocyte culture series (table 41) might give the impression that the female cells were more susceptible to the lytic effects of high calcium ion concentrations than those of the male. Fig.23 was chosen from one of the smears of culture series No.5 (male-versus-female) to show that the incidence of chromosomal breaks was higher in the female cells. The ratios of 1.5 : 1 in chromosomal breaks, 1.4 : 1 in tetraploidy and 1.8 : 1 in endoreduplication were estimated while comparing their incidence in pure female and pure male mixed-lymphocyte cultures respectively. Such results were also confirmed during the karyotyping of the abnormal metaphases of the male-versus-female culture series.

Fig. 23:- Showing a photomicrograph of a metaphase spread prepared from a male-versus-female 110 µg/ml. culture. Note the isochromatid gap of the female cell (arrow) and the normal chromosome complement of the male cell (left).
(Giemsa stain, X 1500).