Results

RESULTS

In this work nearly all the results were collected and presented in table (3).

The 20 cervical carcinoma cases were classified into 3 grades

according to modified Broder's grading method and 4 stages according to International Federation of Gynecologist and Obstetricians (FIGO):(*Wright et al., 1994*)

(1) According to the grade

- grade I   : 3 cases   (15 %) Fig.(15)
- Grade II : 14 cases   (70 %) Fig.(16)
- grade III : 3 cases   (15 %) Fig.(17)

(2) According to the stage:

- stage I   8 cases   (40 %)
- stage II   4 cases   (20%)

Serial No.	Grade	Stage	Age	C	P53	HPV
1	G2	II	59	2.6	-	-ve
2	G2	II	75	3.6	-	+ve, 16 & 18
3	G2	II	62	3.9	-	+ve, 16 & 18
4	G2	IV	43	4.3	-	+ve, 16 & 18
5	G2	I	60	2.5	-	+ve, 31,33 & 35
6	G2	IV	60	4.9	+++	-ve
7	G1	I	58	3.5	+	+ve, 16 & 18
8	G2	III	60	4.1	++	-ve
9	G1	I	65	2.4	-	+ve, 16 & 18
10	G1	I	50	2.5	-	-ve
11	G2	IV	60	4.2	++	-ve
12	G3	I	45	3.4	-	+ve, 31,33 & 35
13	G2	I	45	2.8	-	+ve, 16 & 18
14	G3	I	51	3.2	-	-ve
15	G2	II	60	4.6	-	+ve, 16 & 18
16	G2	III	40	3.2	-	+ve, 16 & 18
17	G3	IV	35	4.3	++	-ve
18	G2	I	60	2.9	-	+ve, 16 & 18
19	G2	III	45	3.9	++	-ve
20	G2	III	33	3.3	-	+ve, 16 & 18

Table (3) : Summary for most of the results.

N.B. : C ≡ average DNA content

In Situ Hybridization Results

I. Control group:

(a) Positive control type I : 4 sections of cervical tissue previously known to be infected with HPV type 16 *(From The Central Medical Academy of Amsterdam, Holland)* showed nuclear purplish blue staining in some epithelial cells. The nuclei of the other tissue cells whether epithelial or stromal appeared pink due to counter stain. These sections showed this hybridization signals only with the (16 + 18) probe mixture. Fig (18 (19) .

(b) positive control type II : Twenty sections (A section for each case). were treated with positive DNA probe which is specific for genomic human DNA Purplish blue stain of high intensity is visible in all the tissue cells whether epithelial or stromal fig (20).

(c) Negative control : Tissues of this group (20 sections) were treated with negative control probe which produces no hybridization signals. Nuclei of this group appeared pink due to the counter stain.

II Cervical squamous cell carcinoma group.

In this study it was found that, 12 of the 20 cases examined showed strong hybridization signal in the form of purplish - blue nuclear staining for high risk HPV in a percentage of 60 %. The percentage of HPV negative tumors was 40% as it was found that no hybridization signal could be detected in the other 8 tumors.

Ten of the 12 HPV positive tumors showed positive hybridization signal for HPV types 16 and 18. Fig. (21) (22) (23) (24) , while DNA of HPV types 31, 33, and 35 was detected in the nuclei of the other 2 tumors Fig. (25) (26). As seen, the percentage of HPV types 16 & 18 detection in cervical carcinoma cases was 50%, while that for HPV types 31, 33, and 35 was 10% only.

All the tumors studied were examined for HPV types 6 and 11 probe mixture (low risk group) but all of them did not show any hybridization signal.

* Correlation between cervical carcinoma grade and HPV status:

The results showed that in grade I tumors 2 cases of the 3 were positive for HPV, in grade II tumors, the HPV positive cases were 9 of 14, while in grade III, the positive tumors were one of 3 table(4).

These results represented an inversely proportionate correlation between cervical carcinoma grade and status of HPV. (r = - 0.19) Fig.(13) But this negative correlation was statistically insignificant (P>0.05).

* Correlation between tumor stage and status of HPV.

In stage one tumors, 6 of 8 cases were HPV positive, in stage II, the positive HPV tumors were 3 of 4, in stage III, 2 of 4 cases were HPV positive and in stage IV, only one case of 4 showed positive

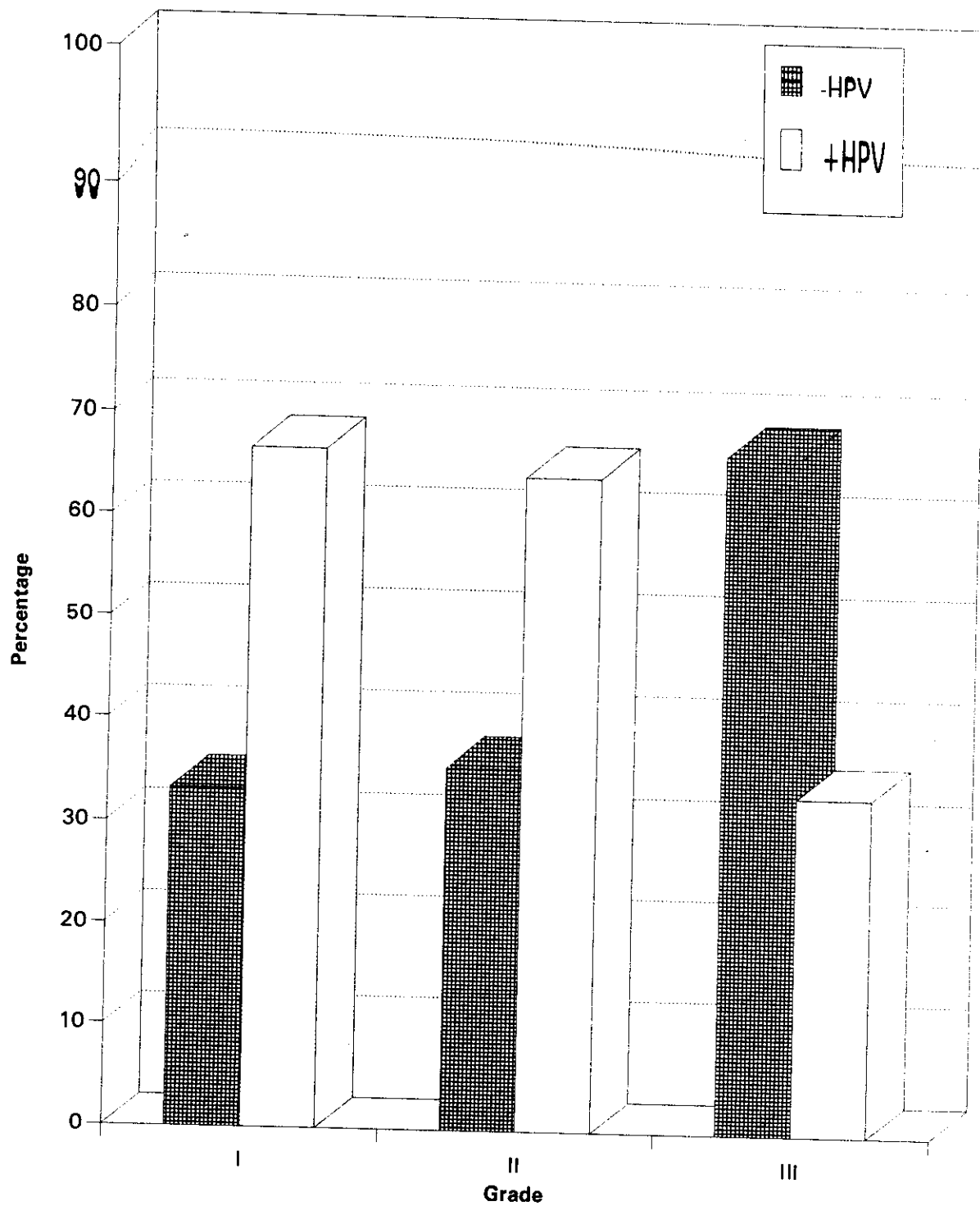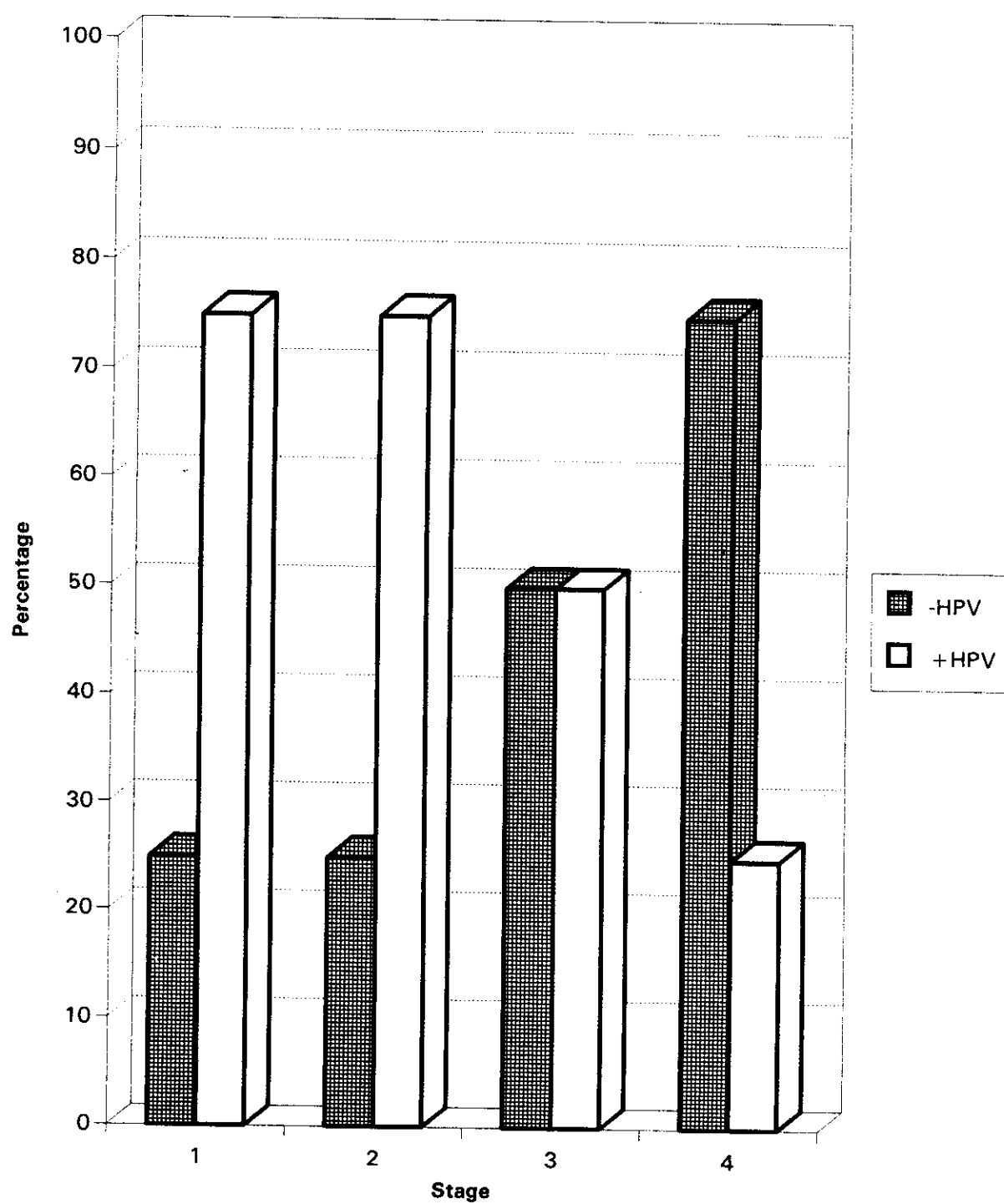# Fig. 13 : Cervical Carcinoma Grade and Status of HPV

Fig. 14 : Cervical Carcinoma Stage and Status of HPV

hybridization signal for HPV. Table (5). These results showed that, the higher the cervical carcinoma stage, the lower the percentage of HPV positive cases (r = - 0.39) Fig.(14) and this negative correlation is statistically significant (P < 0.05).

Grade HPV	GI	GII	GIII	row total
- Ve	1 33.3 %	5 35.7%	2 66.7%	8 40%
+ Ve	2 66.7%	9 64.3%	1 33.3%	12 60%
column Total	3 15%	14 70%	3 15%	20 100%

Table (4) Relationship between tumor grade and HPV status.

Stage HPV	I	II	III	IV	row total
- Ve	2 25 %	1 25%	2 50%	3 75%	8 40%
+ Ve	6 75%	3 75%	2 50%	1 25%	12 60%
column Total	8 40%	4 20%	4 20%	4 20%	20 100%

Table (5) Relationship between tumor stage and status of HPV.
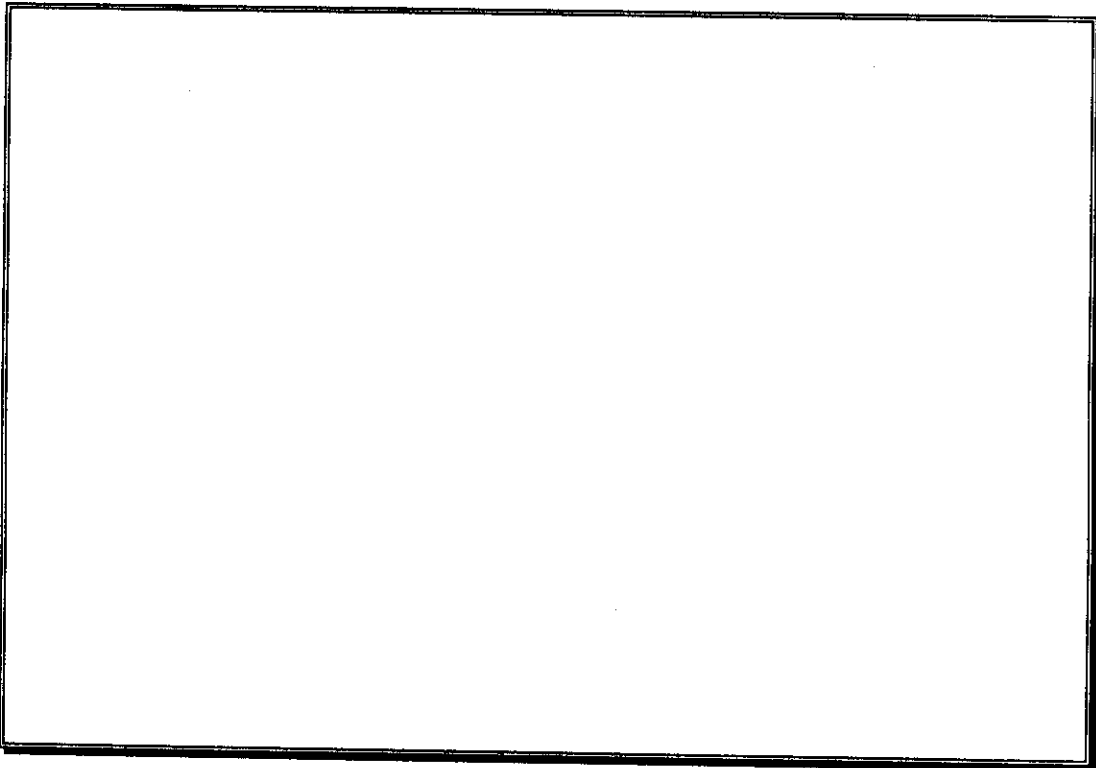
Fig. 15 : Squamous cell carcinoma of the cervix grade I, (H & E x 100)
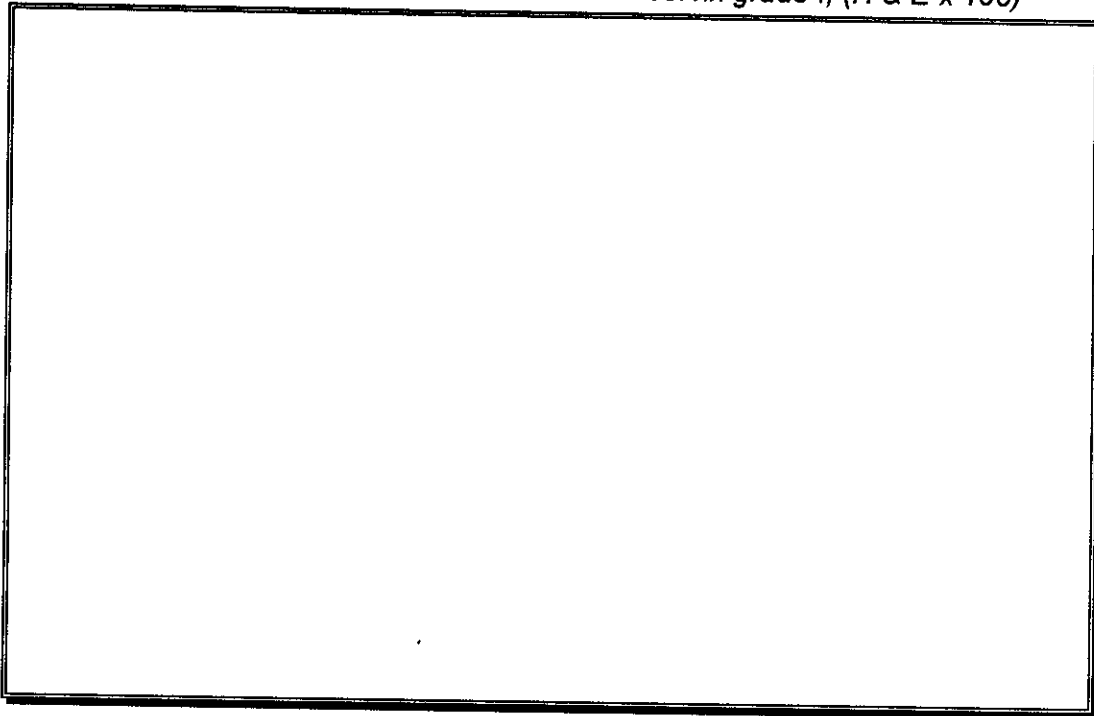
Fig. 16: Squamous cell carcinoma of the cervix grade II, (H & E x 100)
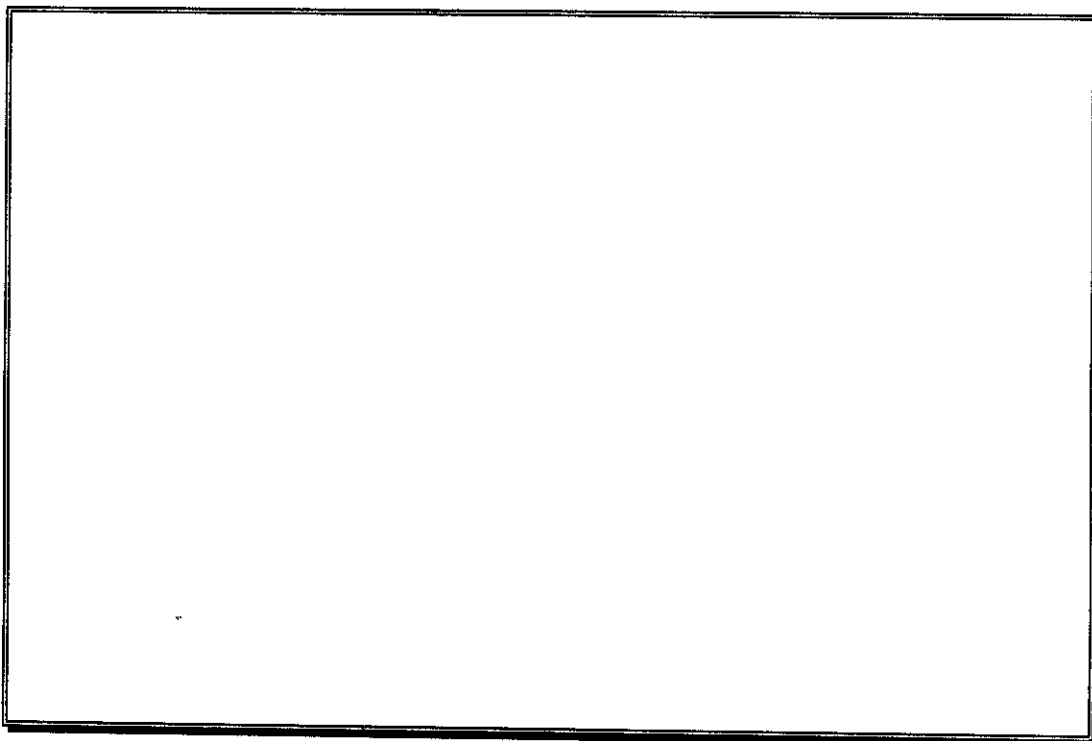
Fig. 17 : Squamous cell carcinoma of the cervix grade III, (H & E x 100)
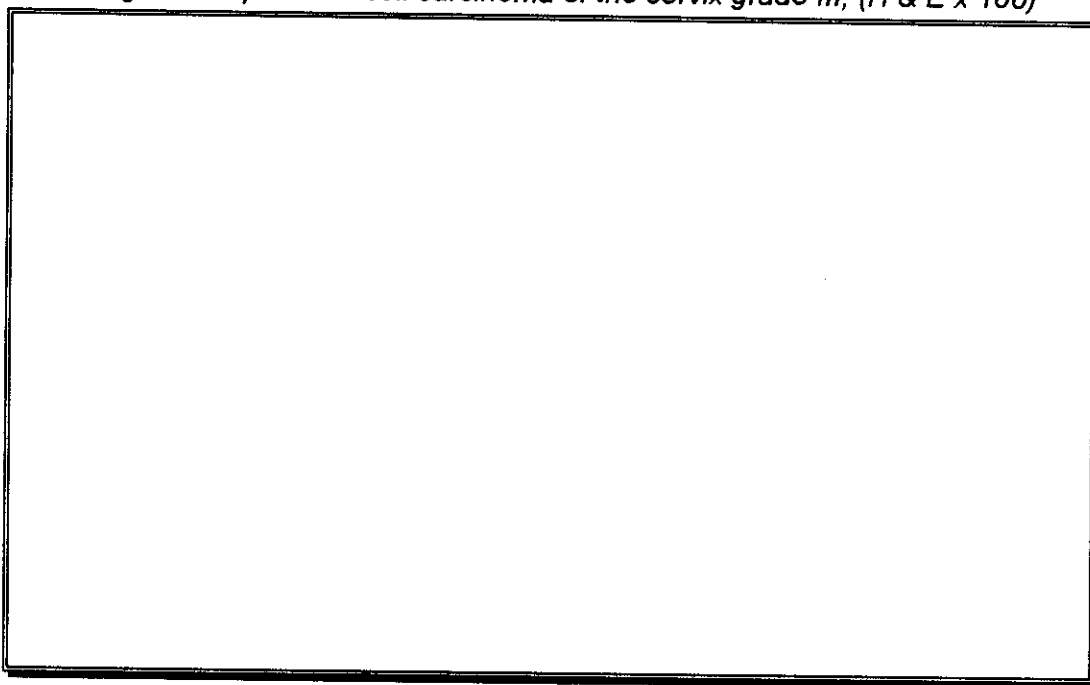

Fig. 18: Positive control type I which showed nuclear purplish blue staining of some nuclei of cervical epithelium other nuclei showed the color of counter stain (In situ hybridization x 100)
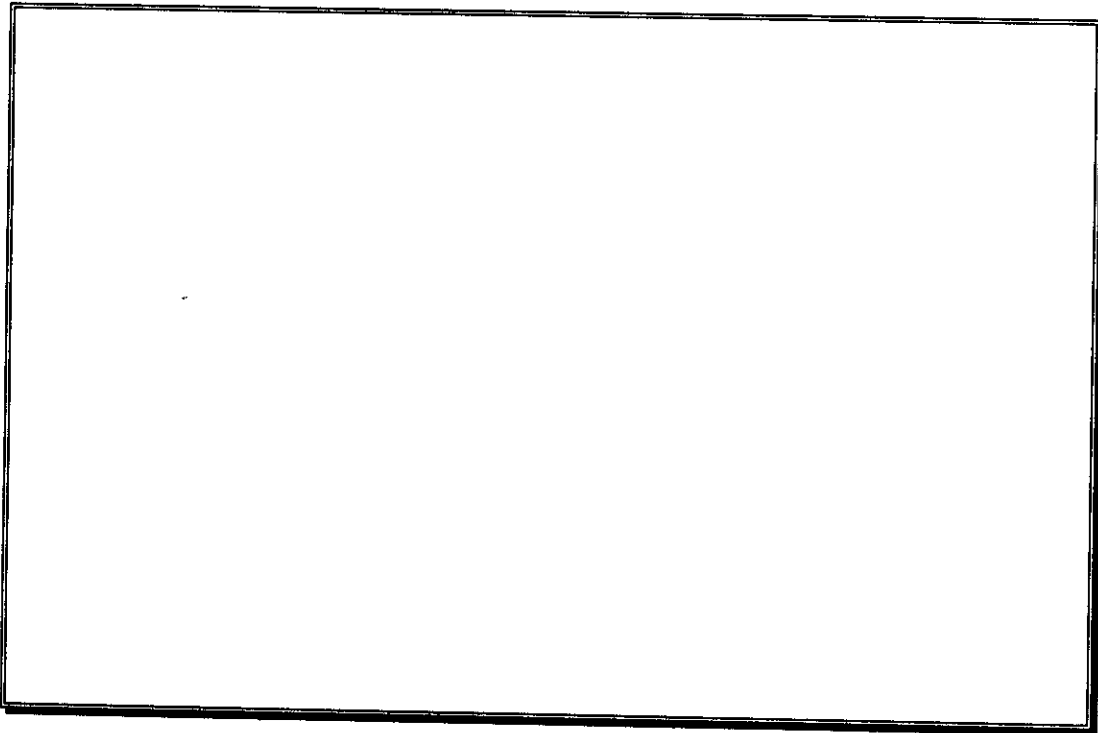
Fig. 19: Higher magnification for the previous one (In situ hybridization x 400)
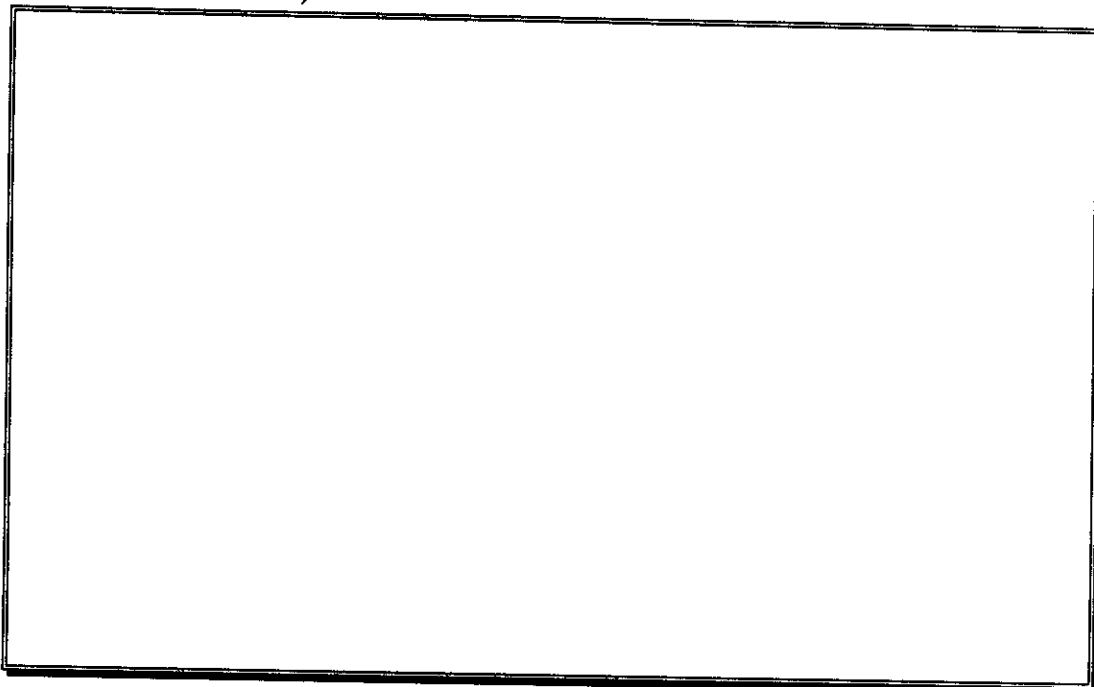
Fig. 20: Positive control type II (Human DNA probe). All the epithelial and stromal nuclei showed positive hybridization signal (In situ hybridization x 400)
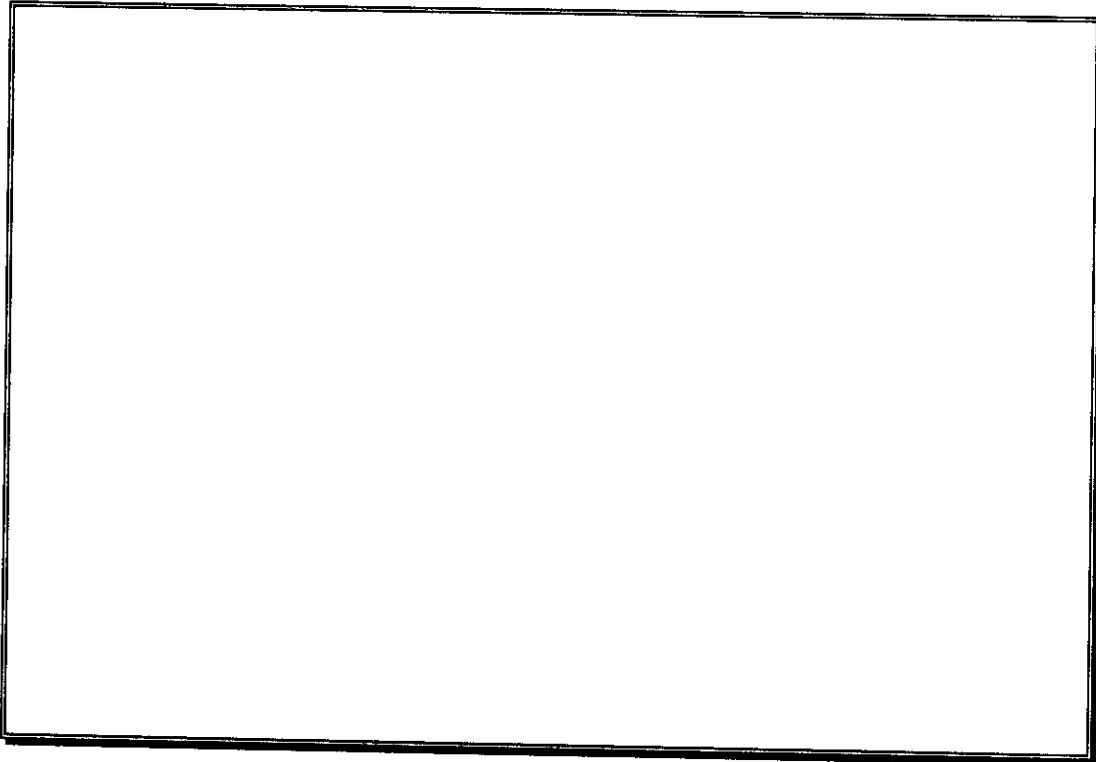
Fig. 21 : Grade I, squamous cell carcinoma of the cervix showing positive hybridization signal for HPV 16 & 18 (In situ hybridization x 100).
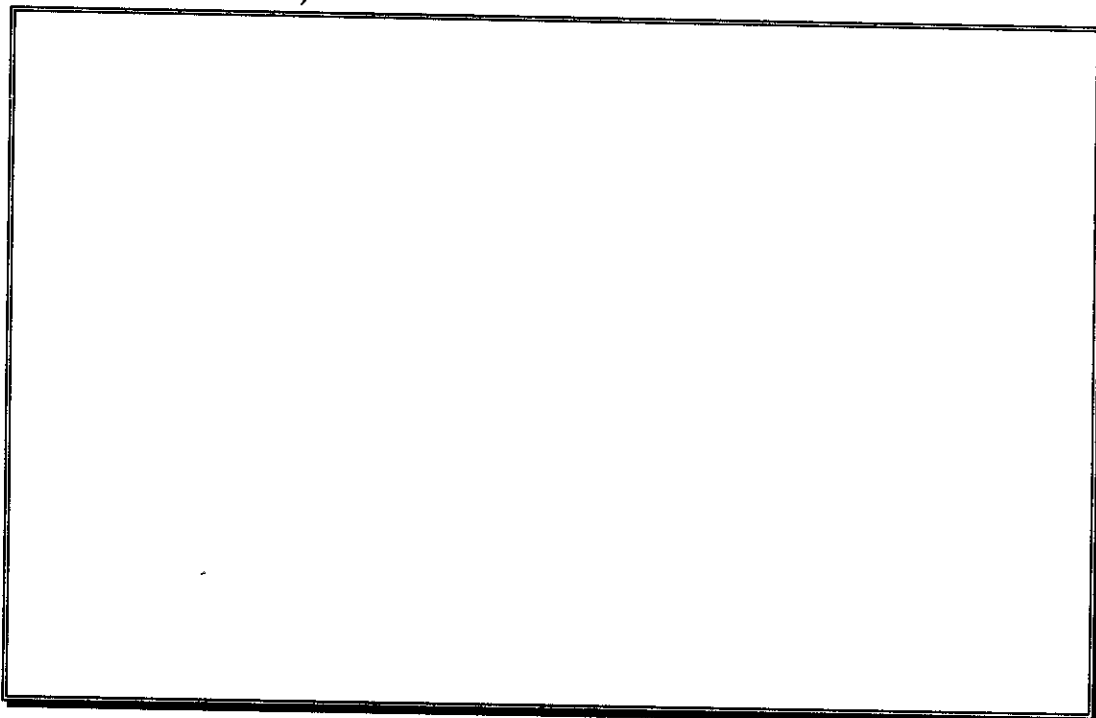

Fig. 22: Higher magnification of the previous one (In situ hybridization x 400)
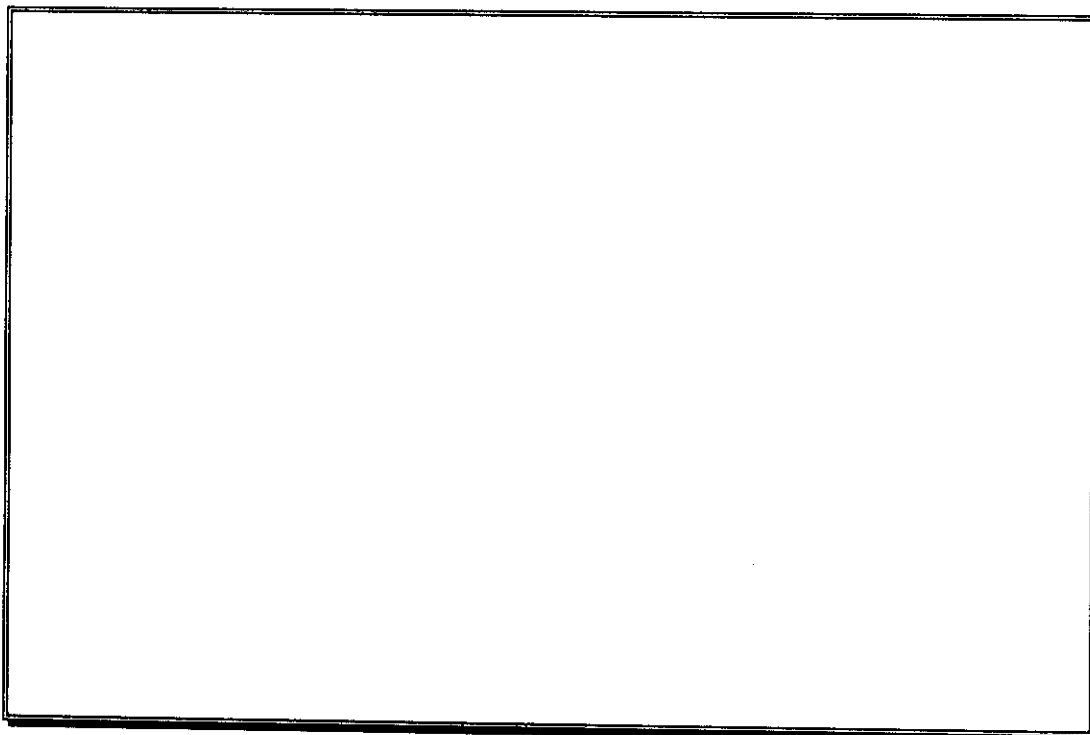
Fig. 23: Squamous cell carcinoma of the cervix showing positive hybridization signal for HPV 16 & 18 (In situ hybridization x 100)
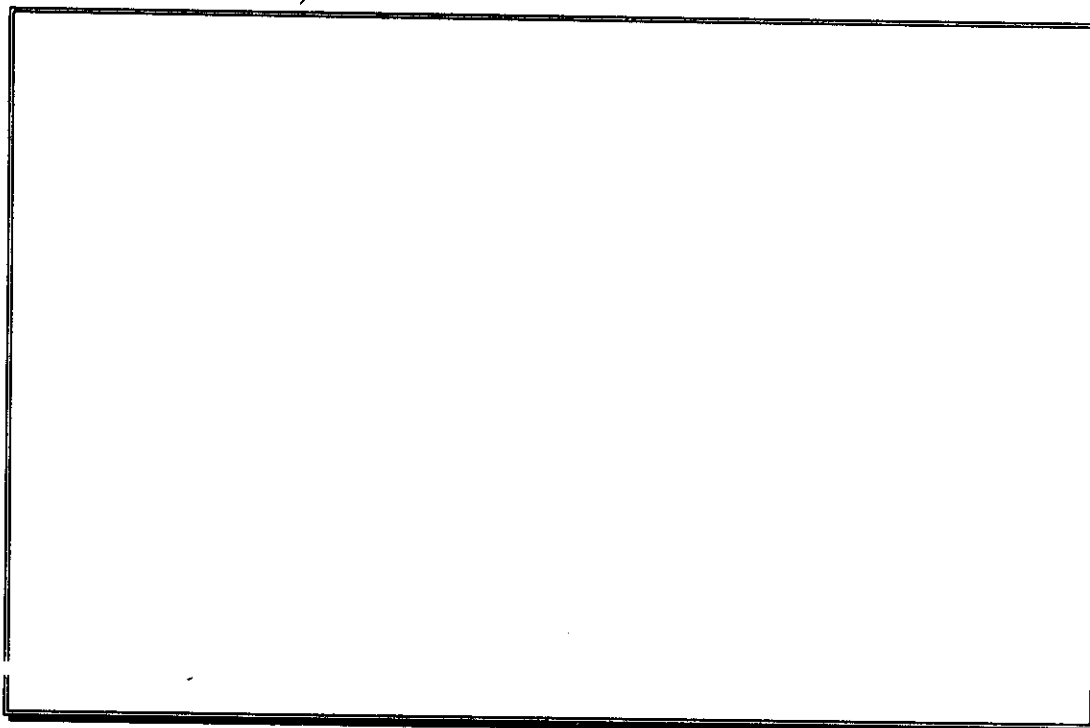

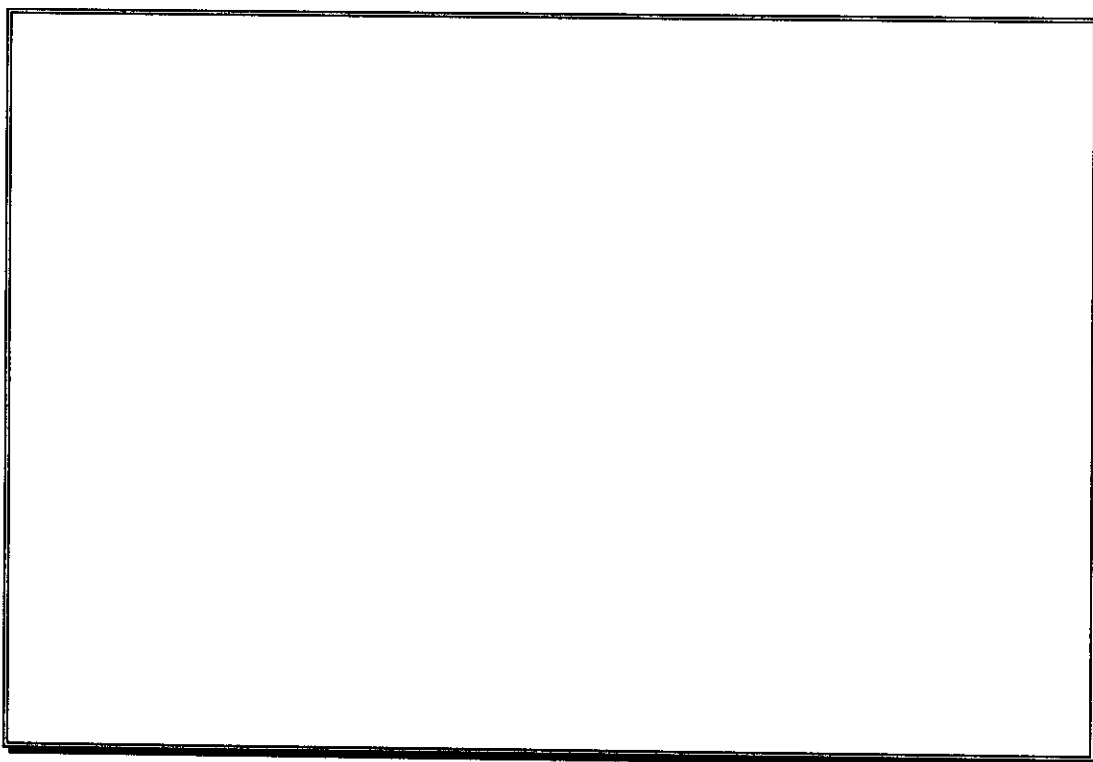Fig. 24: Higher magnification of the previous one (In situ hybridization x 400)

Fig. 25 : Grade II, squamous cell carcinoma of the cervix showing positive hybridization signal for HPV 31, 33 and 35 (In situ hybridization x 250)
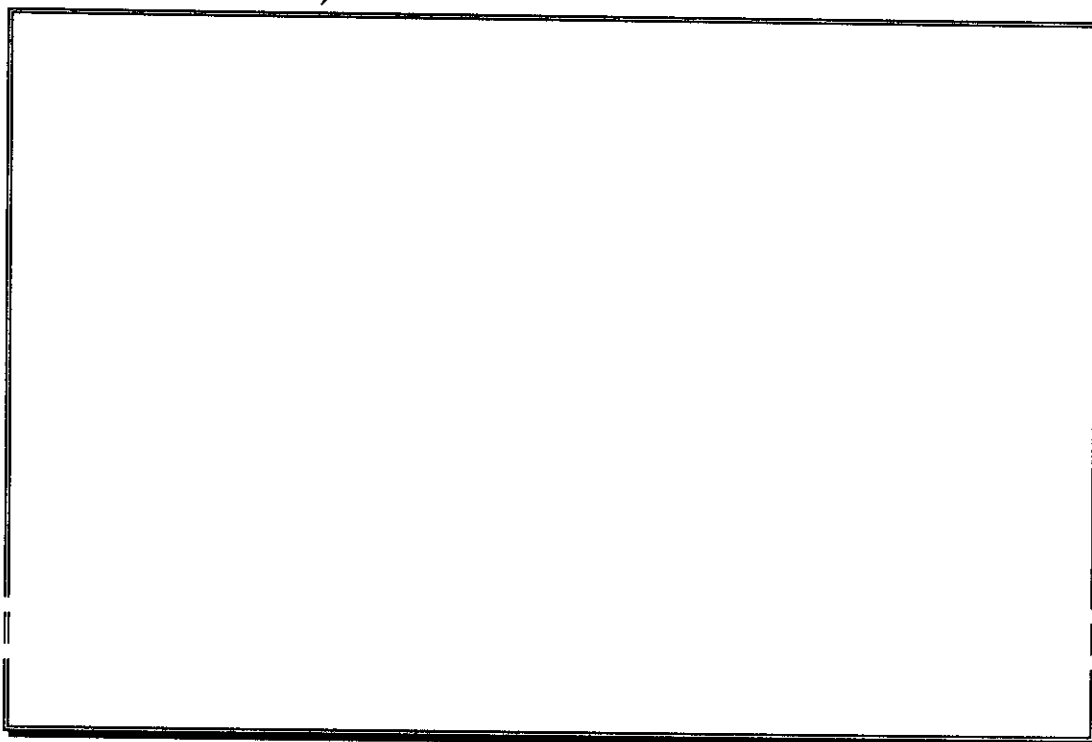

Fig. 26: High magnification of the previous case (In situ hybridization x 400)

P53 Tumor Suppressor Gene Results

I. Control group.

The normal cervical epithelium did not show positive immunostaining for P53.

II. Cervical squamous cell carcinoma cases:-

The immunohistochemical staining was observed in the nucleus, while the cytoplasm failed to be stained. Fig.(29).

As shown in table(3) P53 immunostaining was observed in only 6 of the 20 cases studied in a percentage of 30%. One of the positive cases showed p53 positivity or overexpression in less than 5% of the tumor cells (i.e. +) Fig. (30). P53 oncoprotein was detected in (5-50%) of tumor cells in 3 cases (+ +) Fig. (31) (32) and in more than 50% of tumor cells in 2 cases (+ + +) Fig (33) & (34).

* Correlation between P53 immunostaining results and cervical carcinoma grade:

In the studied cases of this work the percentage of p53 positive cases in grade I tumors was 33.3%, in grade II it was 28.6 %, while in cases of grade III it was 33.3% table (6). These results showed no specific correlation between tumor grade and status of p53. Fig.(27) r= Zero and p=1.

* Correlation between cervical carcinoma stage and status of p53.

In this study, stage I, tumors showed p53 overexpression in one case out of 8 (12.5%), stage II, the 4 cases studied showed negative p53 immunostaining, in stage III cases, 2 out of 4 cases showed p53 overexpression (50%), while in stage IV, 3 of the 4 tumors studied showed positive p53 immunostaining in a percentage of 75% table(7).

These results revealed that; the higher the cervical carcinoma stage, the higher the number of cases showed p53 overexpression. This positive correlation (r= 0.54) Fig. (28) is statistically significant (p = 0.013).

* Correlation between p53 and HPV status.

The results of this work showed that, all p53 positive cases were found to be negative for high risk HPV except only one case which was positive for both p53 and HPV. Also all the p53 negative cases showed positive hybridization signals for high risk HPV except only 3 cases which showed negative results for both HPV and p53 table (8). These results showed an inversely proportionate correlation between p53 overexpression and detection of high risk HPV. This correlation (r = - 0.58) is highly significant (p = 0.007).
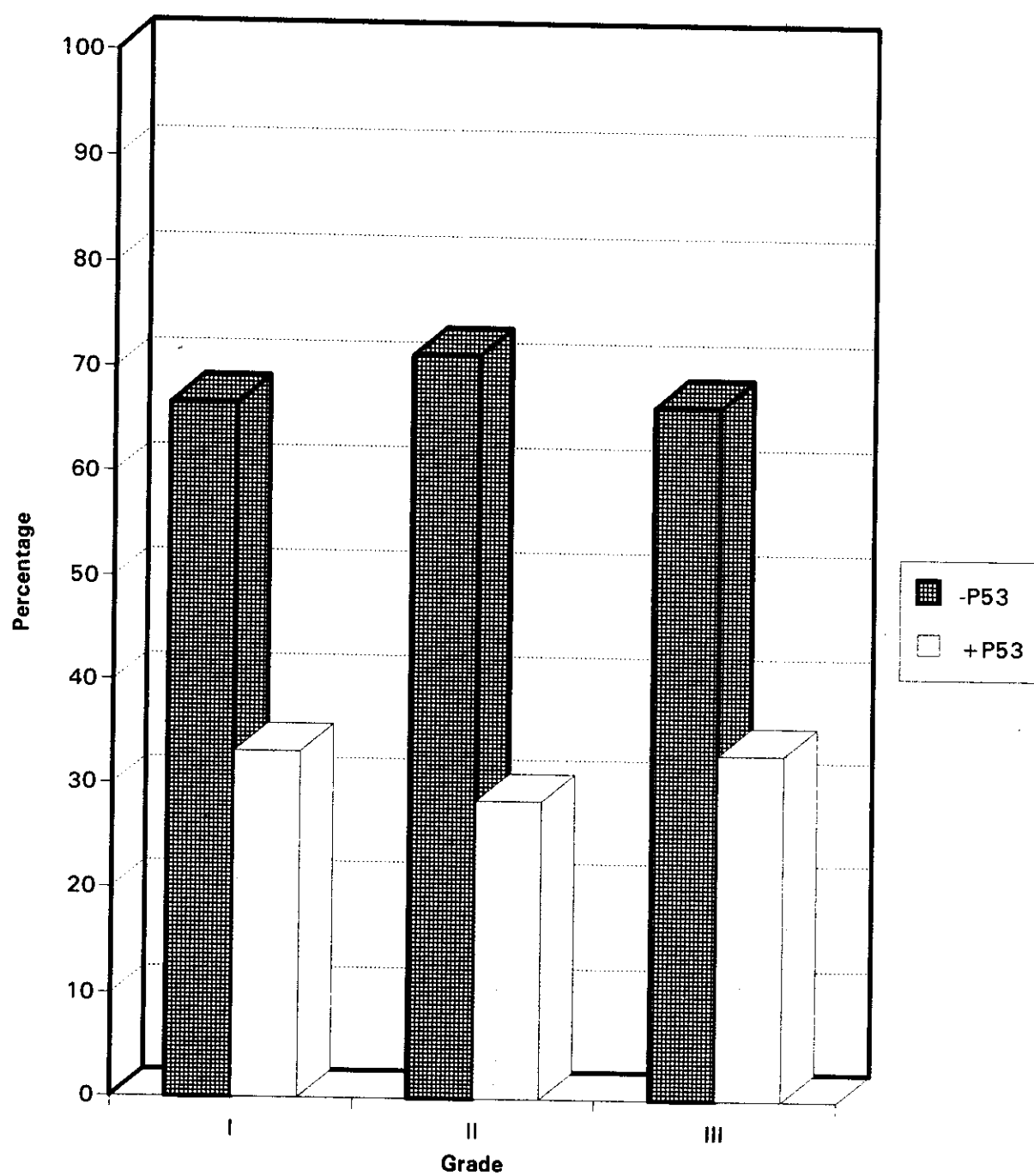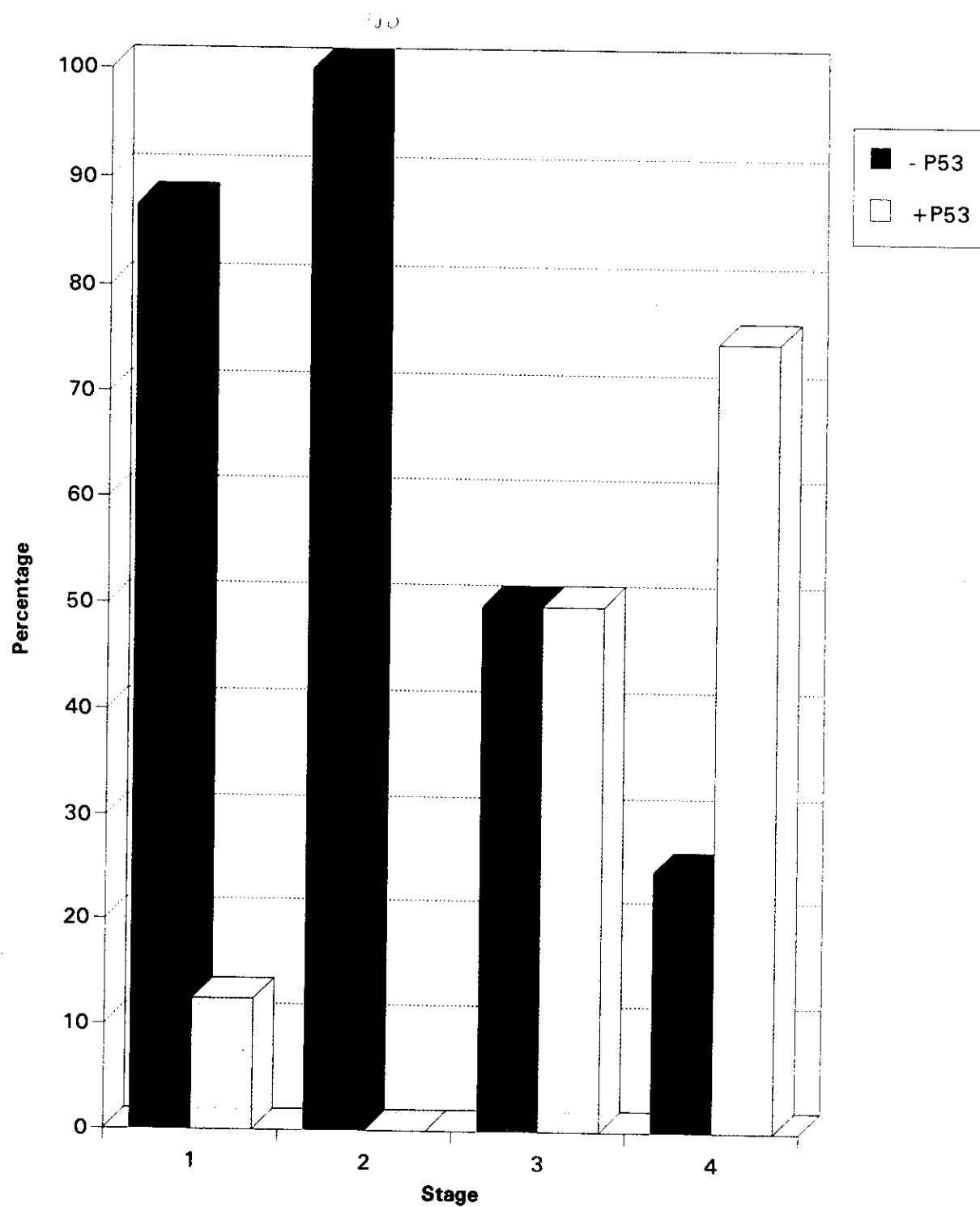
Fig. 27 : Cervical Carcinoma Garde and Status of P53

Fig. 28 : Cervical Carcinoma Stage and Status of P53

Grade p 53	GI	GII	GIII	row total
- Ve	2 66.7 %	10 71.4%	2 66.7%	14 70%
+ Ve	1 33.3%	4 28.6%	1 33.3%	6 30%
column Total	3 15%	14 70%	3 15%	20 100%

Table (6) Relationship between tumor grade and status of P53

Stage p 53	I	II	III	IV	row total
- Ve	7 87.5 %	4 100%	2 50%	1 25%	14 70%
+ Ve	1 12.5%	0 0%	2 50%	3 75%	6 30%
column Total	8 40%	4 20%	4 20%	4 20%	20 100%

Table (7) Relationship between tumor stage and p53 status.

HPV p 53	- VE	+ Ve	row total
- Ve	3 37.5 %	11 91.3%	14 70%
+ Ve	5 62.5%	1 8.3%	6 30%
column Total	8 40%	12 60%	20 100%

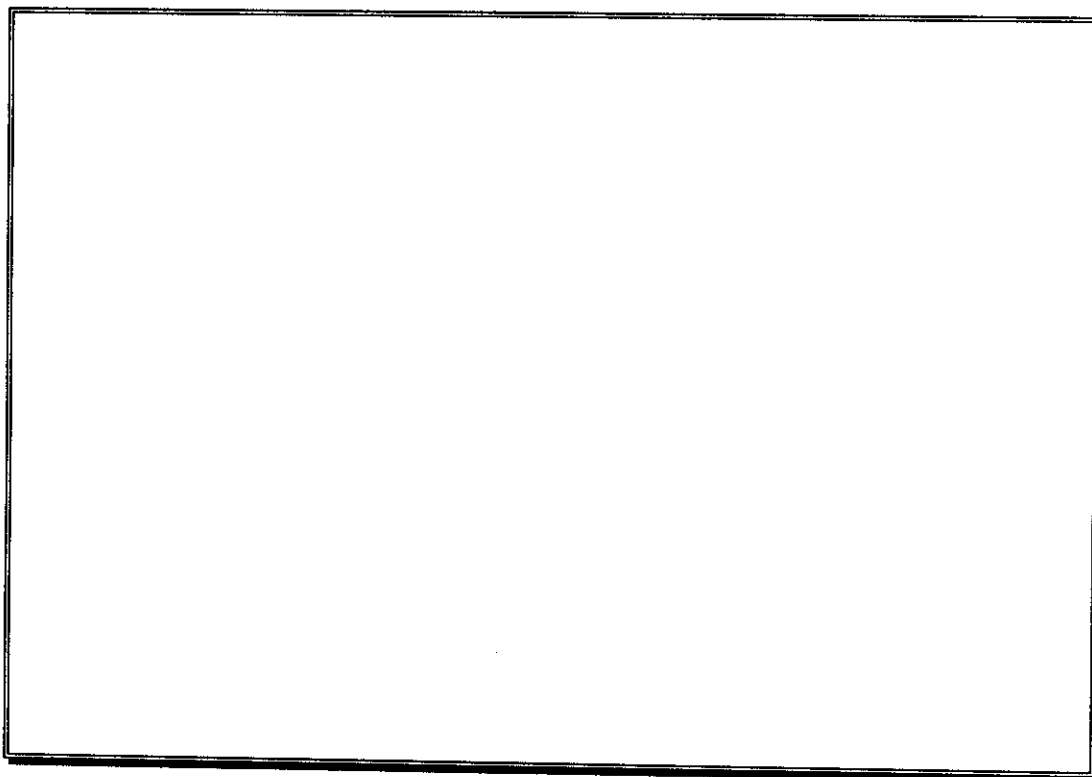*Table (8) Relationship between status of p 53 and HPV*

Fig. 29 : Squamous cell carcinoma of the cervix showing brownish nuclear P53 immunostaining (PAP x 400)
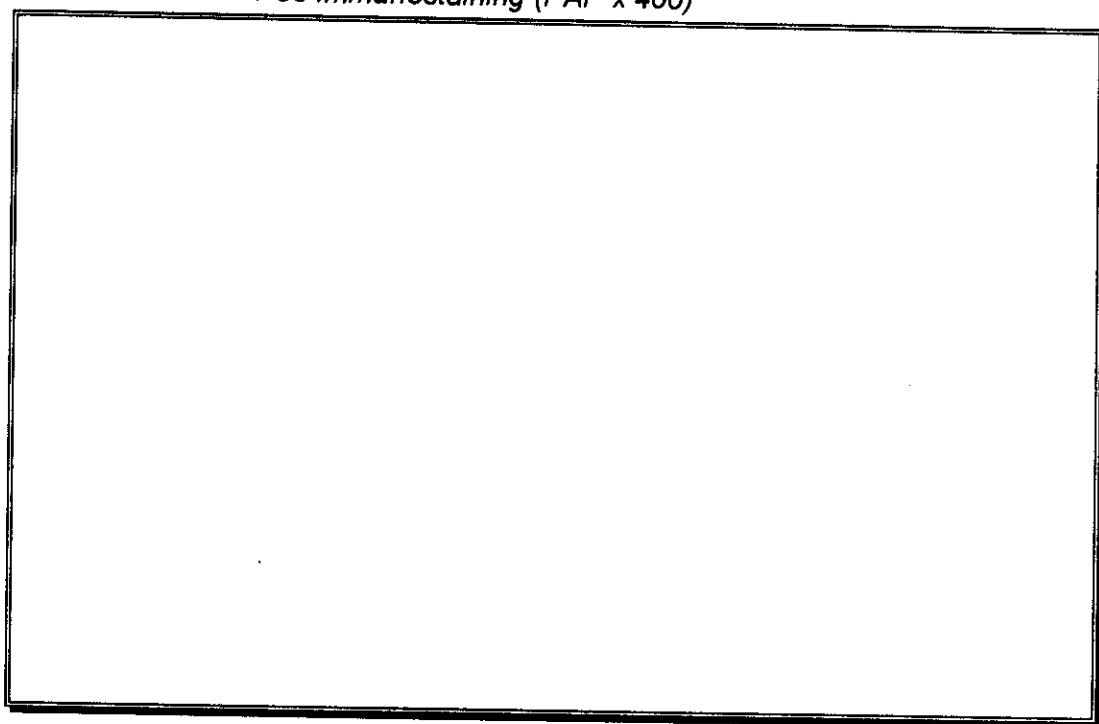

Fig. 30: Squamous cell carcinoma of the cervix with positive P53 immunostaining in less than 5% of the nuclei (PAP x 400).
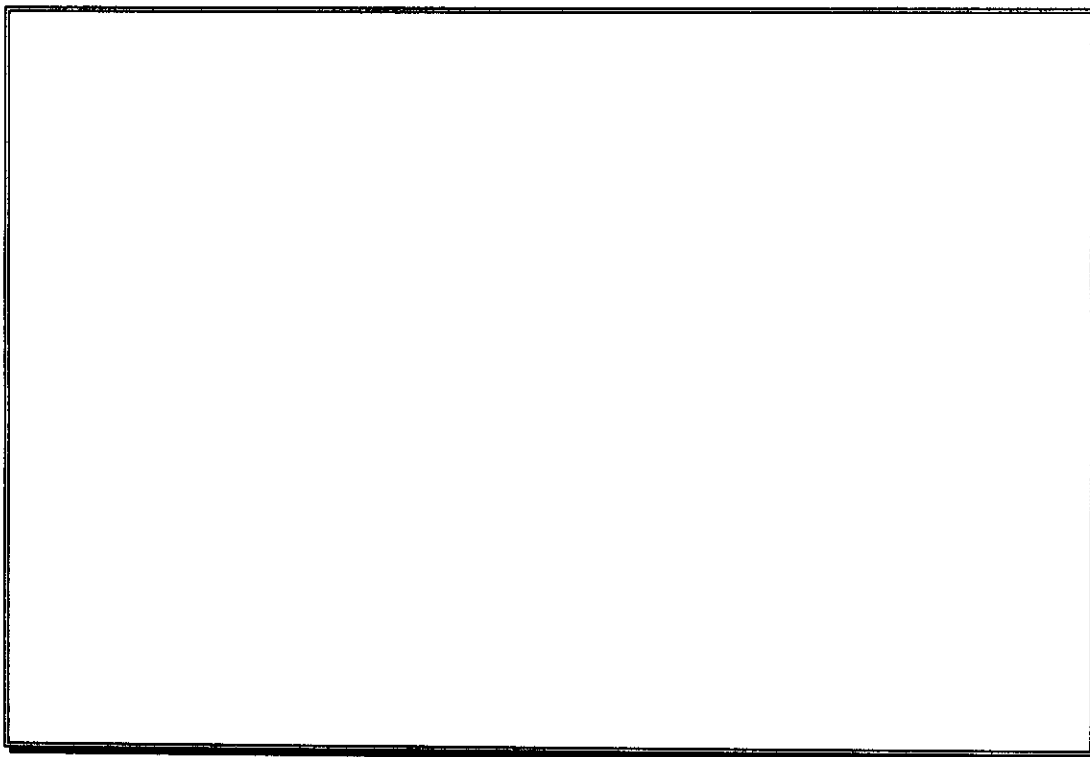
Fig. 31: Squamous cell carcinoma of the cervix with positive P53 immunostaining in 5-50% of the nuclei (PAP x 400)
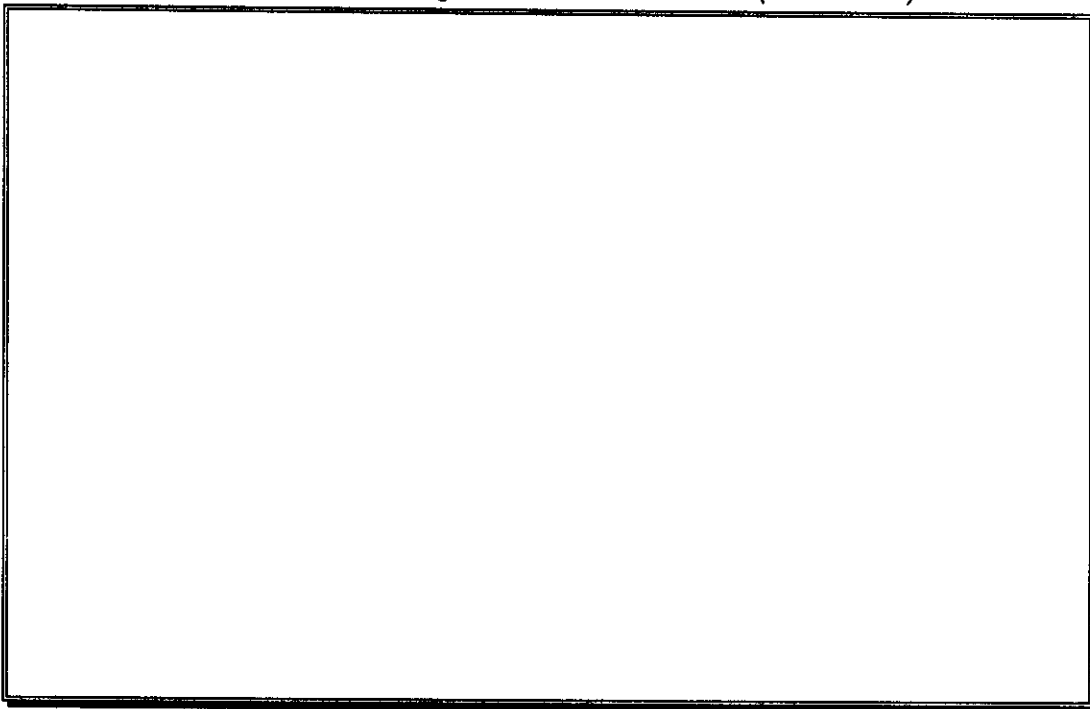

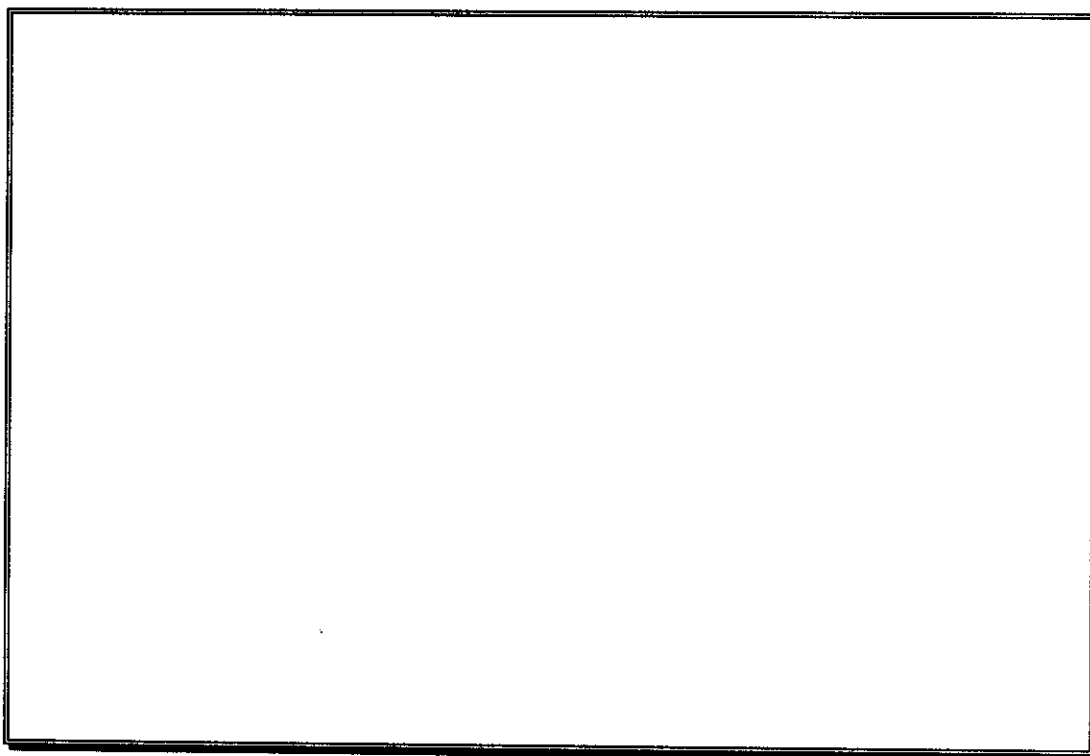*Fig. 32: Higher magnification of the previous case(PAP x 1000)*

*Fig. 33 : Squamous cell carcinoma of the cervix with positive P53
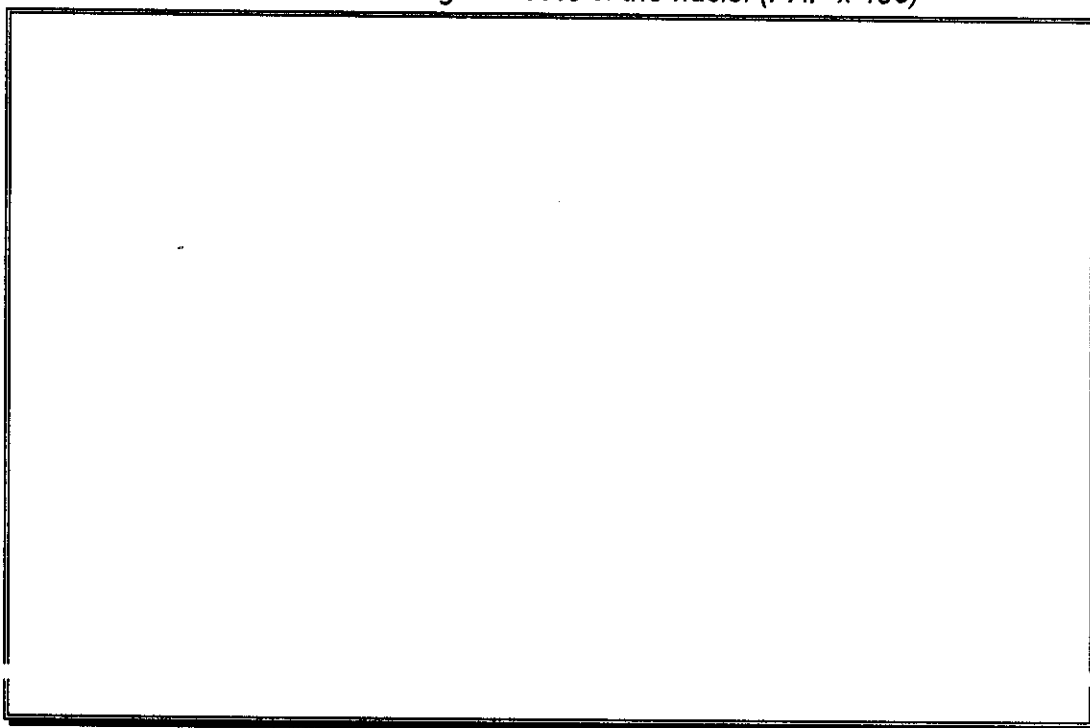immunostaining in > 50% of the nuclei (PAP x 400)*

Fig. 34: Higher magnification of the previous case(PAP x 1000)

DNA Cytophotometric Results

(1) Control group:

The DNA content of the normal control group is showed in Fig.(35) where it shows that, DNA content of the majority of cells were found in the diploid range.

(2) The cervical carcinoma group.

DNA histograms for all the cases were done and classified into 3 types.

(1) Type I histogram	: 6 cases (30%) .	Fig. (36)
(2) Type II histogram	: 10 cases (50%)	Fig. (37)
(3) Type III histogram	: 4 cases (20%)	Fig. (38)

Also the DNA ploidy and aneaploidy pattern and average DNA content for every case was calculated and compared to other results leading to many correlations which are.

* Correlation between ploidy pattern and tumor grade:

Comparing the DNA ploidy pattern results and cervical carcinoma grade, it was found that there is no significant correlation between ploidy pattern and grade of cervical carcinoma ($p > 0.05$). Table (9).
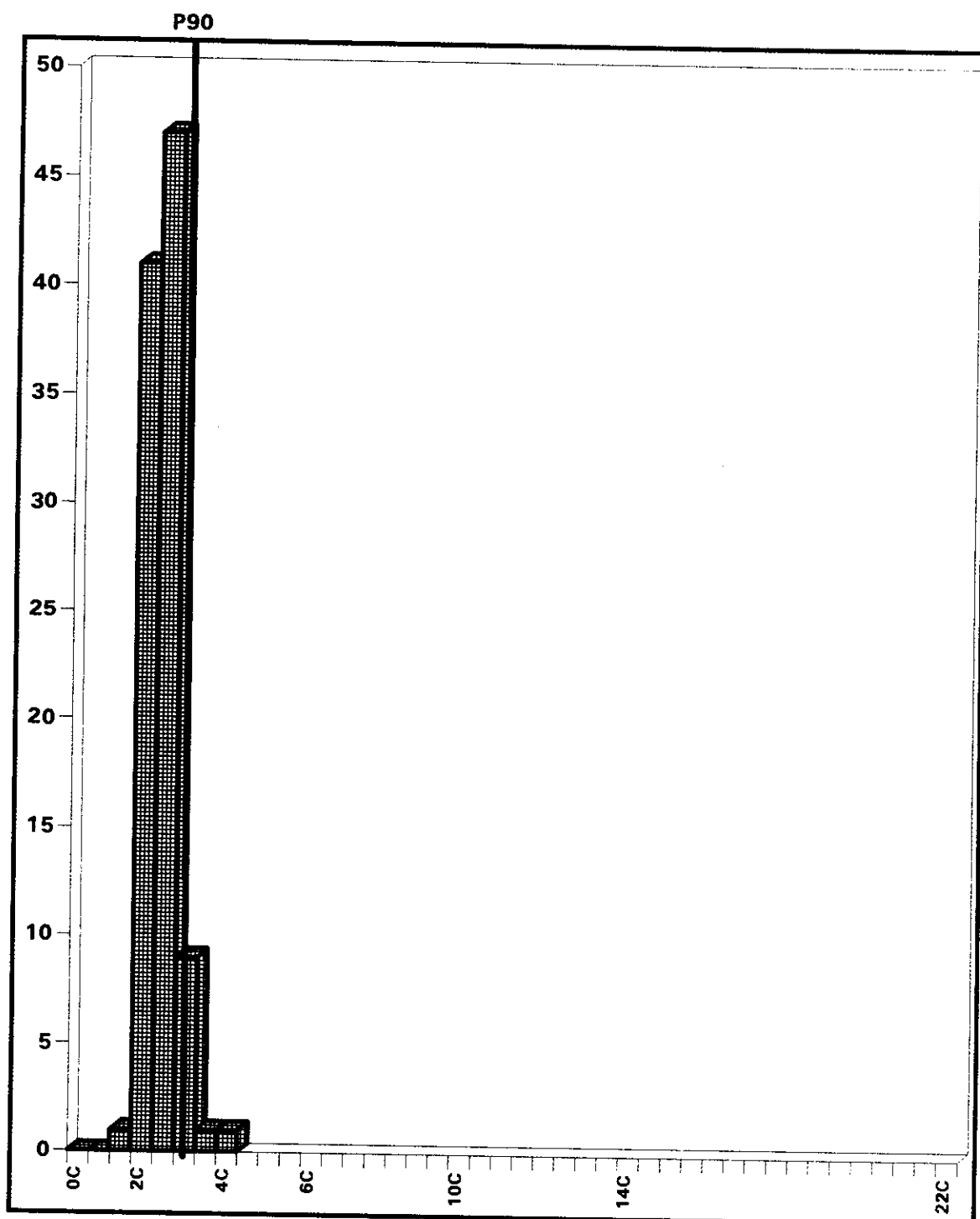
Fig. 35 : DNA Distribution in normal controls
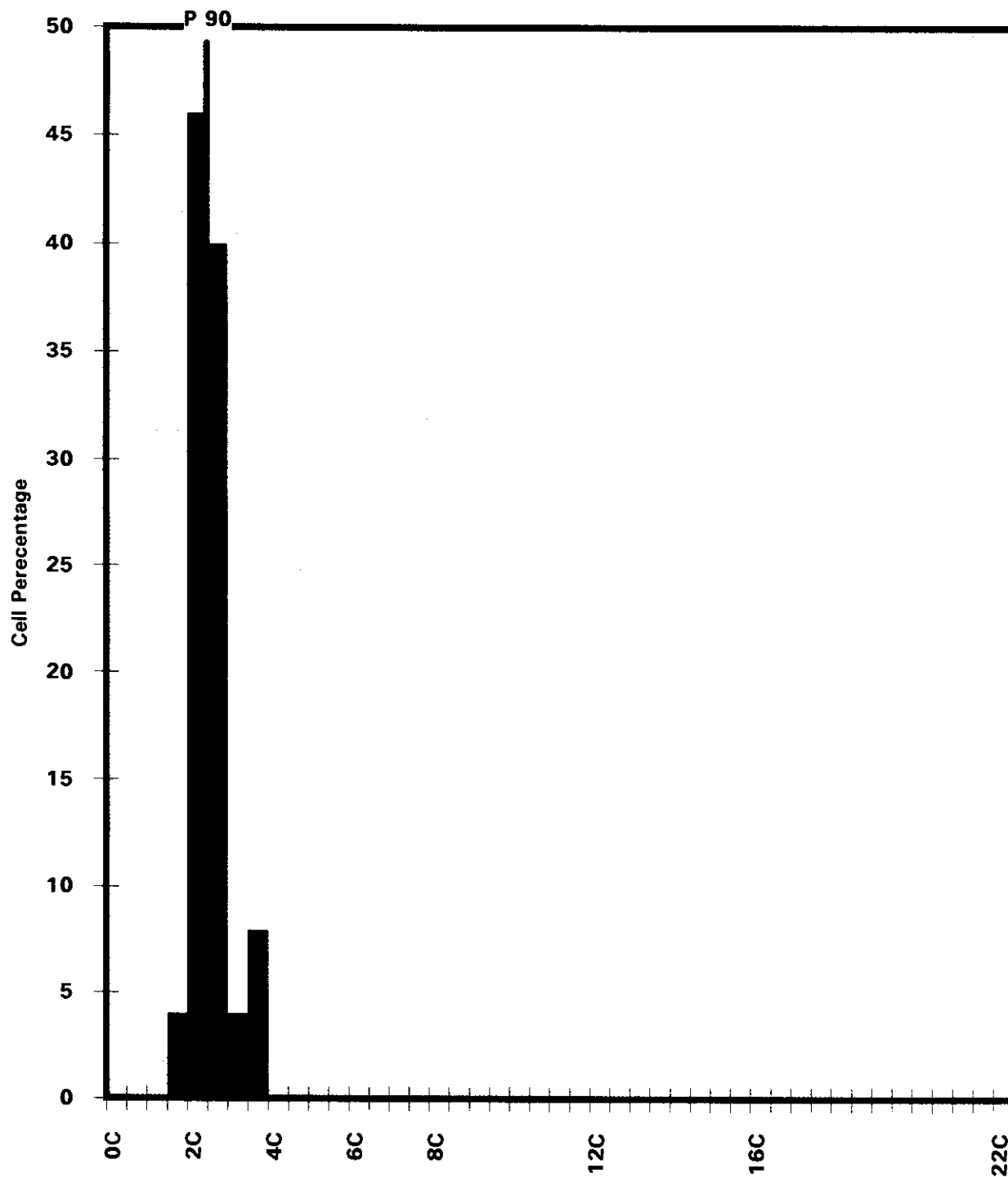
Fig. 36 : Example for histogram type I (Case No.15)

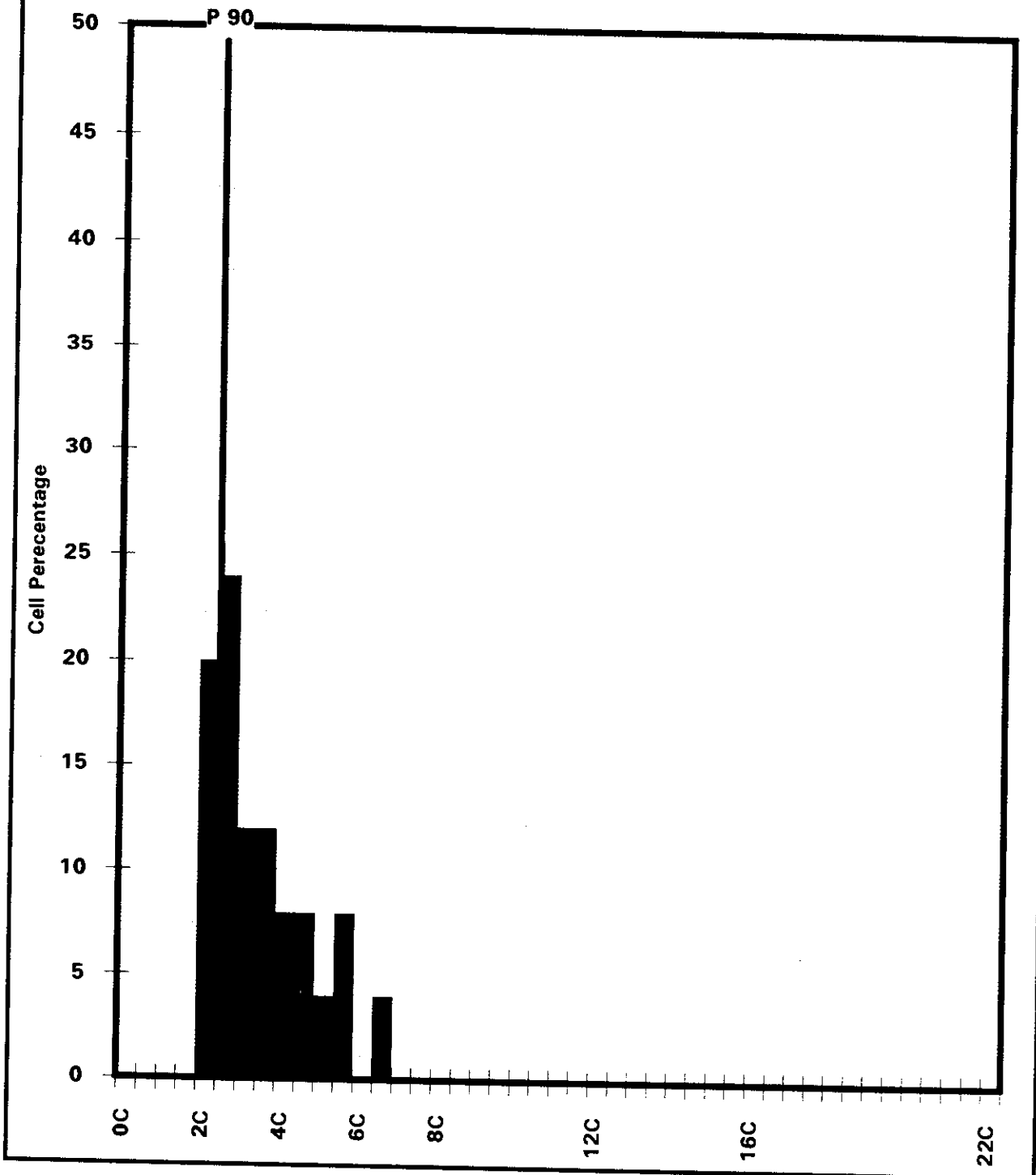Fig. 37 : Example for histogram type II (Case No.8)

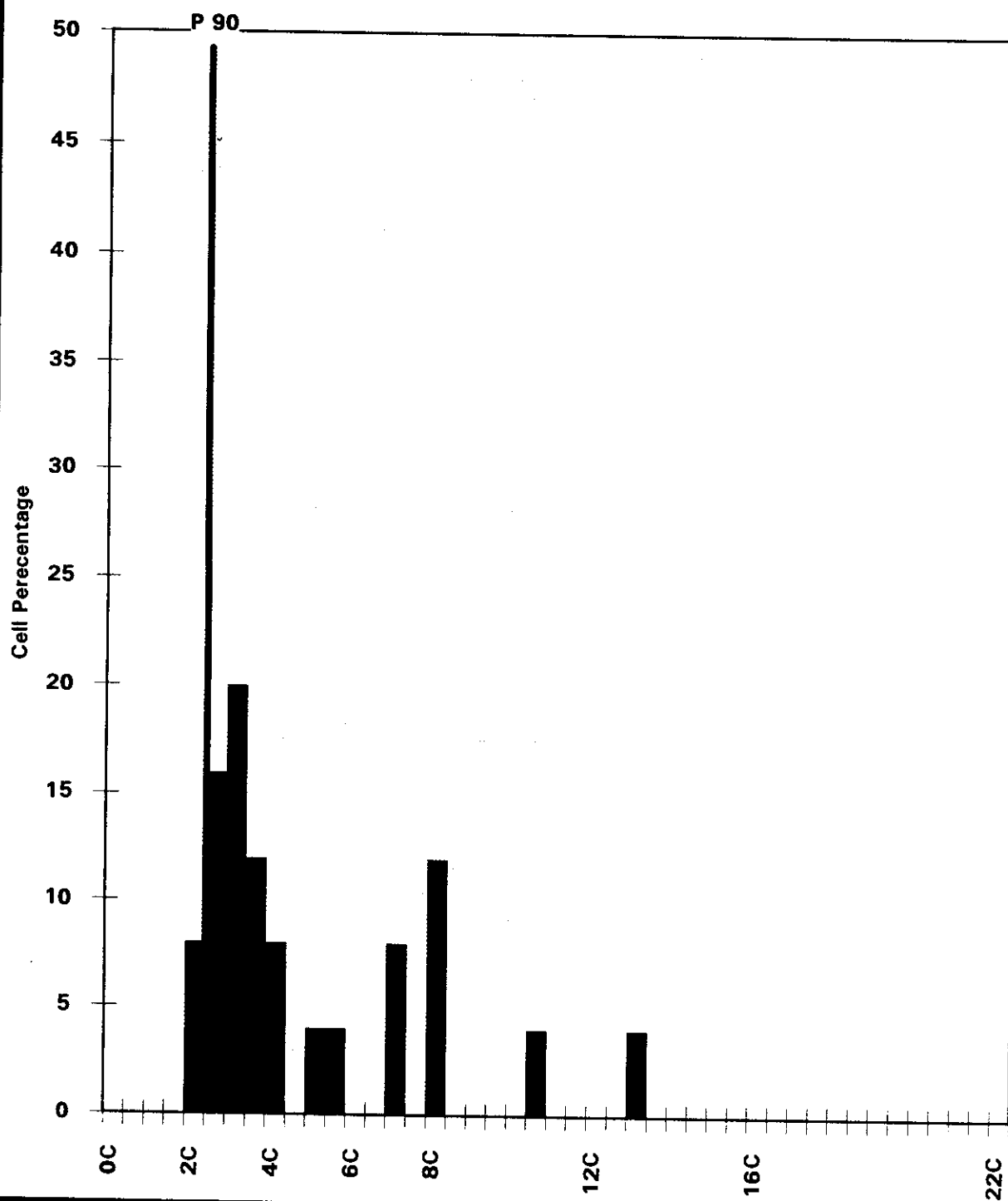Fig. 38 :Example for histogram type III (Case No.20)

Ploidy Grade	Diploid	Aneuploid	Row total
I	2 10%	1 5%	3 15%
II	4 20%	10 50%	14 70%
III	1 5%	2 10%	3 15%
Column total	7 35%	13 65%	20 100%

Table (9):Relationship between ploidy pattern and tumor grade.

* Correlation between average DNA content and cervical carcinoma grade:

The results showed that, in grade I cervical carcinoma 66.7% have (2-3c) content and 33.3% have (3 - 4c) content. In grade II, 28.6% of the tumors have (2-3c), (35.7%) have (3-4c) and 35.7% have (> 4c) while in grade III, 66.7% of the cases have (3-4c) and 33.3 % have (>4c) table (10). Although these results is directly proportional to the cervical carcinoma grade, this positive correlation (r = 0.35) is insignificant (p = 0.13).Fig. (39).

Fig. 39 : Average DNA Content and Cervical Carcinoma Grade
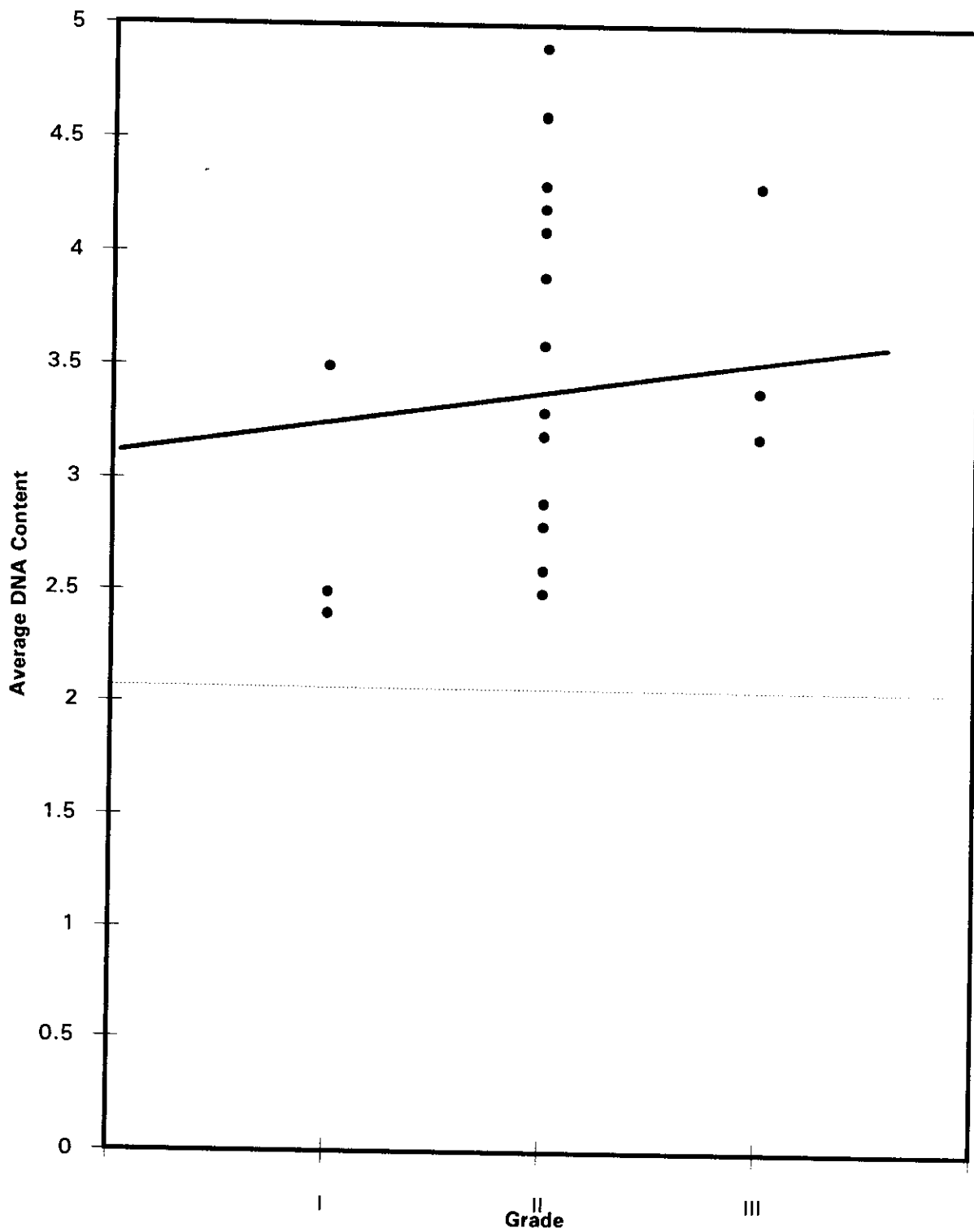
Grade C	GI	GII	GIII	row total
2 - 3c	2 66.7 %	4 28.6%	0 0%	6 30%
3 - 4c	1 33.3%	5 35.7%	2 66.7%	8 40%
> 4c	0 0%	5 35.7%	1 33.3%	6 30%
column Total	3 15%	14 70%	3 15%	20 100%

Table (10) Relationship between tumor grade and average DNA content.

* Correlation between average DNA content and status of HPV.

The comparison between average DNA content of every case and HPV in situ hybridization results (table 11) showed a directly proportionate correlation ($r = 0.28$), but this correlation is statistically insignificant. $p = 0.26$ Fig.(40).

* Histogram of + ve HPV cases group:

Eighty percent of the cells had DNA content under 4c. The other cells showed DNA values ranged from 4 c to 9 c, but the number of cells surpassed 6c are less than 10% thus, this histogram is considered as type II histogram. Fig.(41)

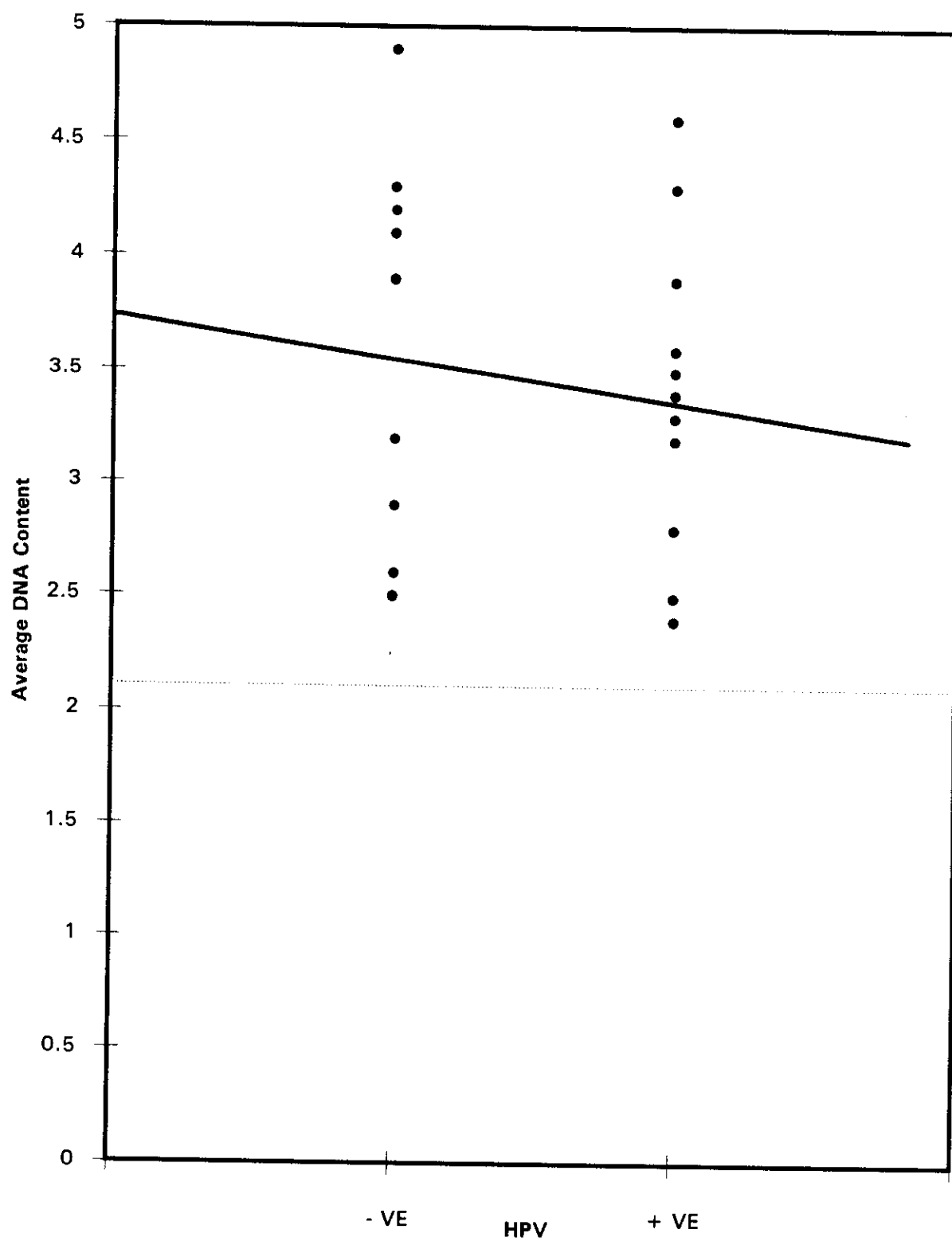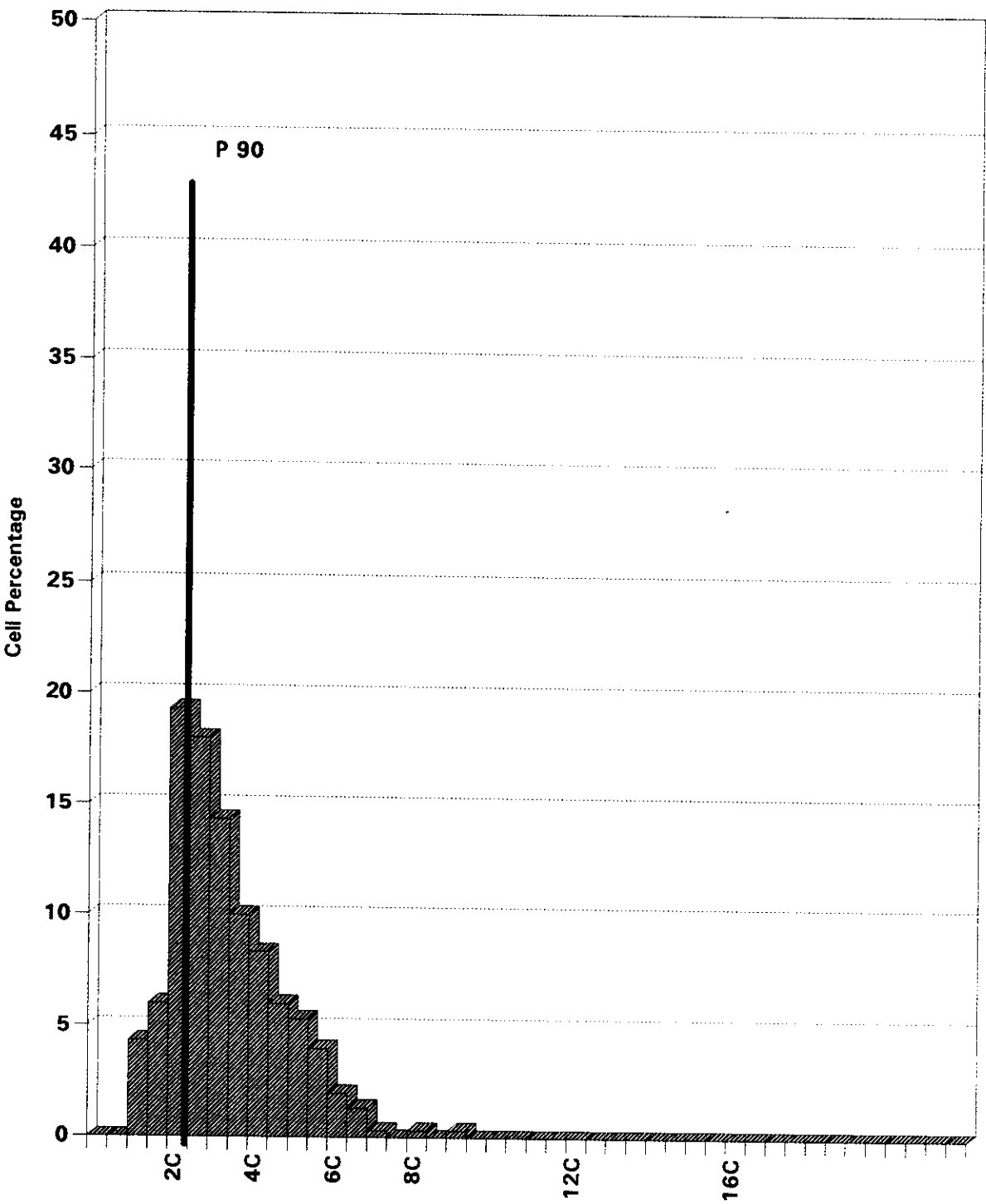## Fig. 40 : Average DNA Content and Status of HPV

Fig. 41 : DNA Distribution of Positive Cases for High Risk
Human Papillomavirus

* Histogram of - ve HPV cases groups:

The cells of this group showed a broadly scattering histogram and the individual DNA content reach levels over 12c. The proportion of the cells over 6c are more than 10%. This group of tumors represented type III histogram. Fig.(42).

HPV C	- ve	+ ve	row total
2 - 3c	2 25 %	4 33.3%	6 30%
3 - 4c	2 25 %	6 50%	8 40%
> 4c	4 50%	2 16.7%	6 30%
column Total	8 40%	12 60%	20 100%

Table (11) relationship between Average DNA content and status of HPV.

* Correlation between the average DNA content and status of P53.

The results showed that, in p 53 negative cases, 42.6 % of the tumors have (2-3c), 42.6% contain (3-4c) and 14.8% contain (> 4c) content. In tumors showed p53 overexpression 33.3% of cases contain (3-4c) and 66.7% have (> 4c) content. table (12)

These results showed that, the average DNA content is directly proportional to the p53 overexpression. This positive correlation (r=0.56) is statistically of high significance (p= 0.009) Fig.(43).

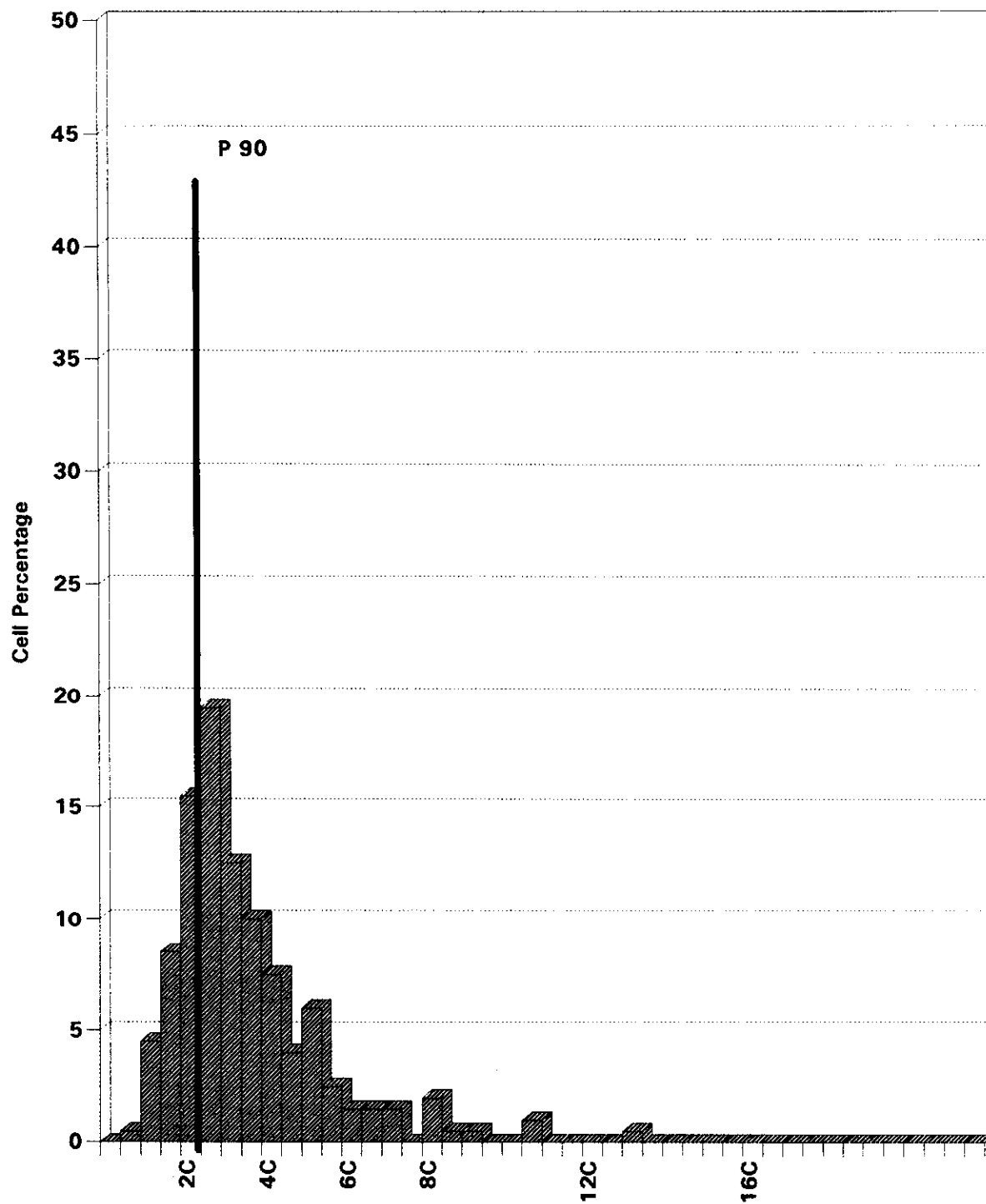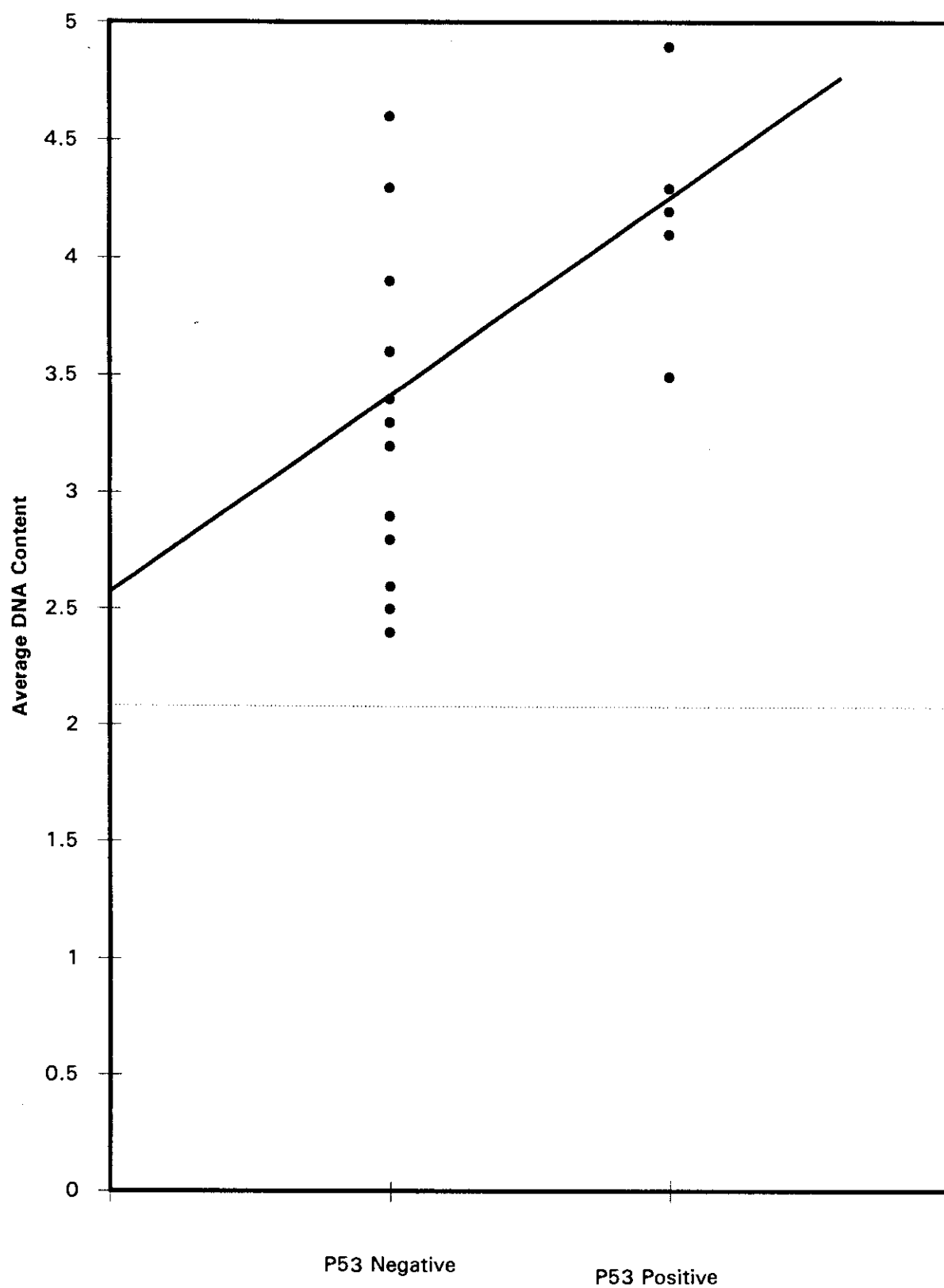Fig. 42   : DNA Distribution of Negative Cases for High Risk
Human Papillomavirus

Fig. 43 : Average DNA Content and Status of P53

* Histogram of + ve p53 cases :

Histogram of this group is comparable to that of the - ve HPV case group but with little shift to the right. Most of the cells are above 3c and the levels of DNA content ranged from 3c to more than 12c The proportion of cells above 6c are more than 10%. Thus, it is type III histogram. Fig.(44).

* Histogram of - ve p 53 cases group:

The cells of this group showed a histogram with about 70% of the cells below 4c and the levels of DNA content of the remaining cells ranged from 4c to even more than 8c But the percentage of cells over than 6c did not surpassed 10% of cells. Thus p53 - ve cases surpassed histogram is type II. Fig.(45).

p 53 C	- ve	+ ve	row total
2 - 3c	6 42.6 %	0 0%	6 30%
3 - 4c	6 42.6 %	2 33.3%	8 40%
> 4c	2 14.8%	4 66.7%	6 30%
column Total	14 70%	6 30%	20 100%

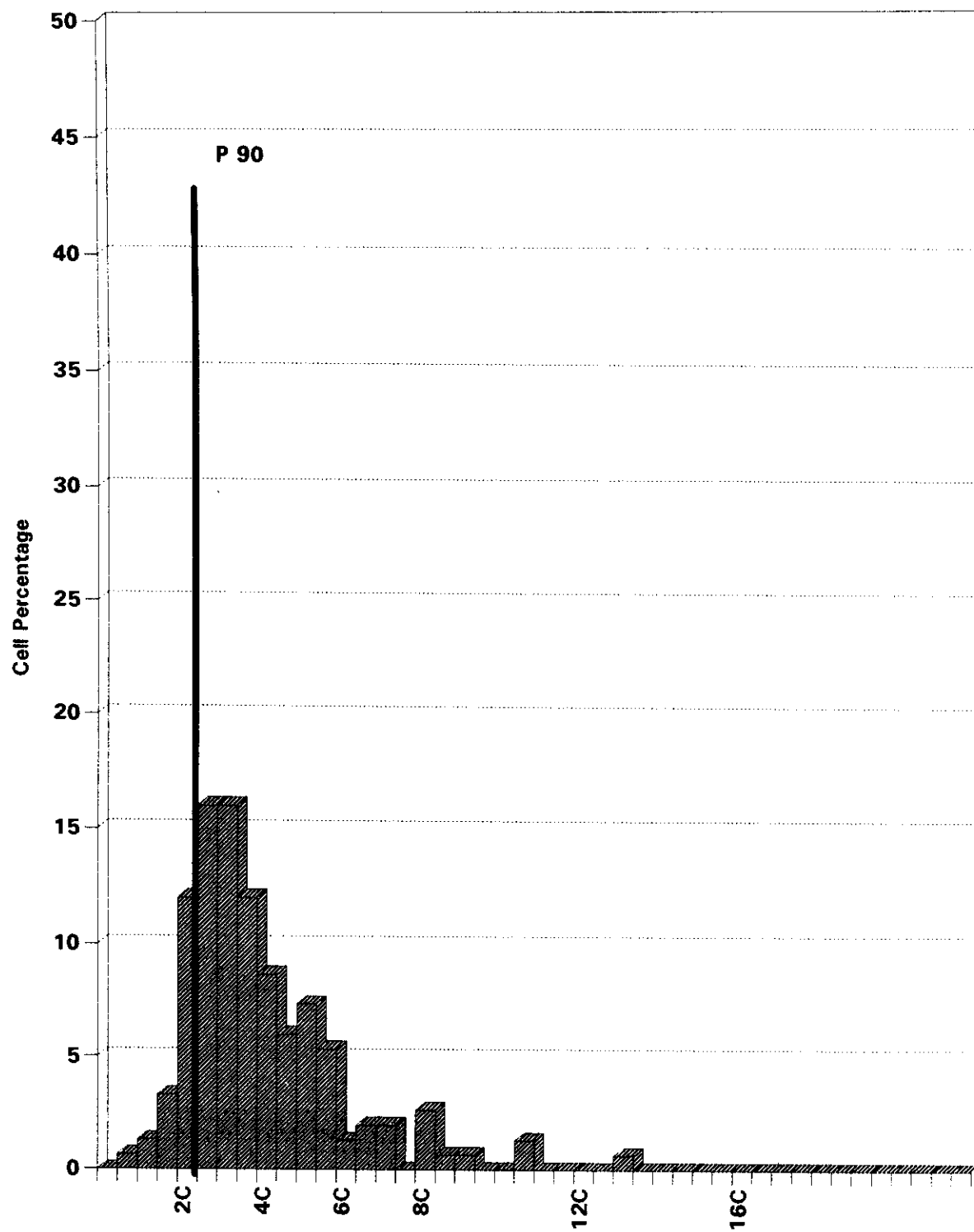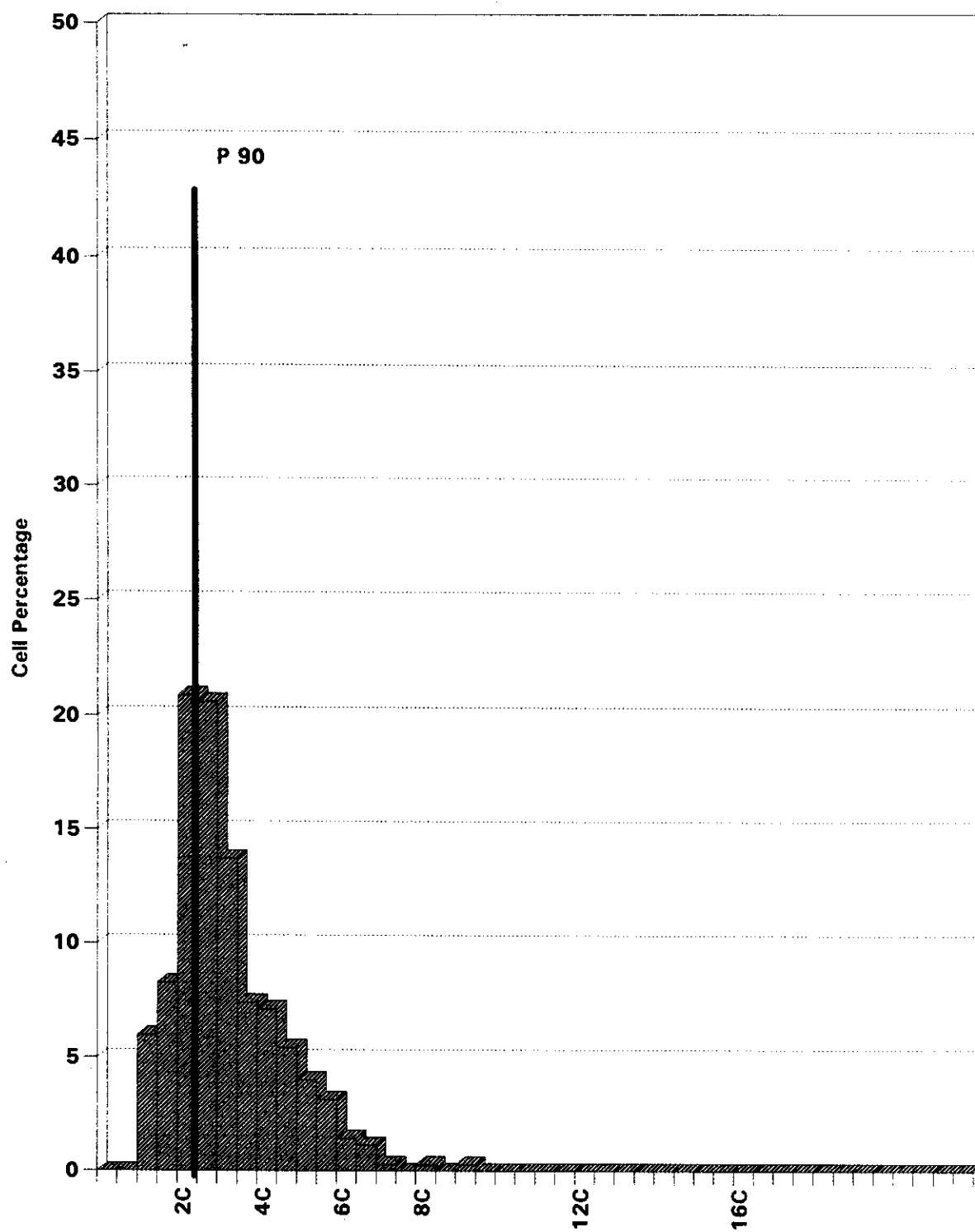*Table (12) relationship between p53 status and average DNA content*

Fig. 44 : DNA Distribution of Positive Cases for P53

Fig. 45   : DNA Distribution of Negative Cases for P53

* **Correlation between ploidy pattern and cervical carcinoma stage.**

Comparing the DNA ploidy pattern and cervical carcinoma stage it was found that there is no significant correlation between diploidy and tumor stage (P>0.05) table(13).

Ploidy pattern Stage	Diploid	Aneuploid	Row total
I	4 20%	4 20%	8 40%
II	1 5%	3 15%	4 20%
III	2 10%	2 10%	4 20%
IV	0 0%	4 20%	4 20%
Column total	7 35%	13 65%	20 100%

Table (13) : Relationship between ploidy pattern and tumor stage.

Correlation between average DNA content and tumor stage.

Comparing the results of average DNA content and cervical carcinoma stage in table (14) showed that, the higher the tumor stage, the higher the average DNA content. This positive correlation (r = 0.77) is of high significance , (p = 0.008).Fig.(46).

Fig. 46 : Cervical Carcinoma Stage and Average DNA Content
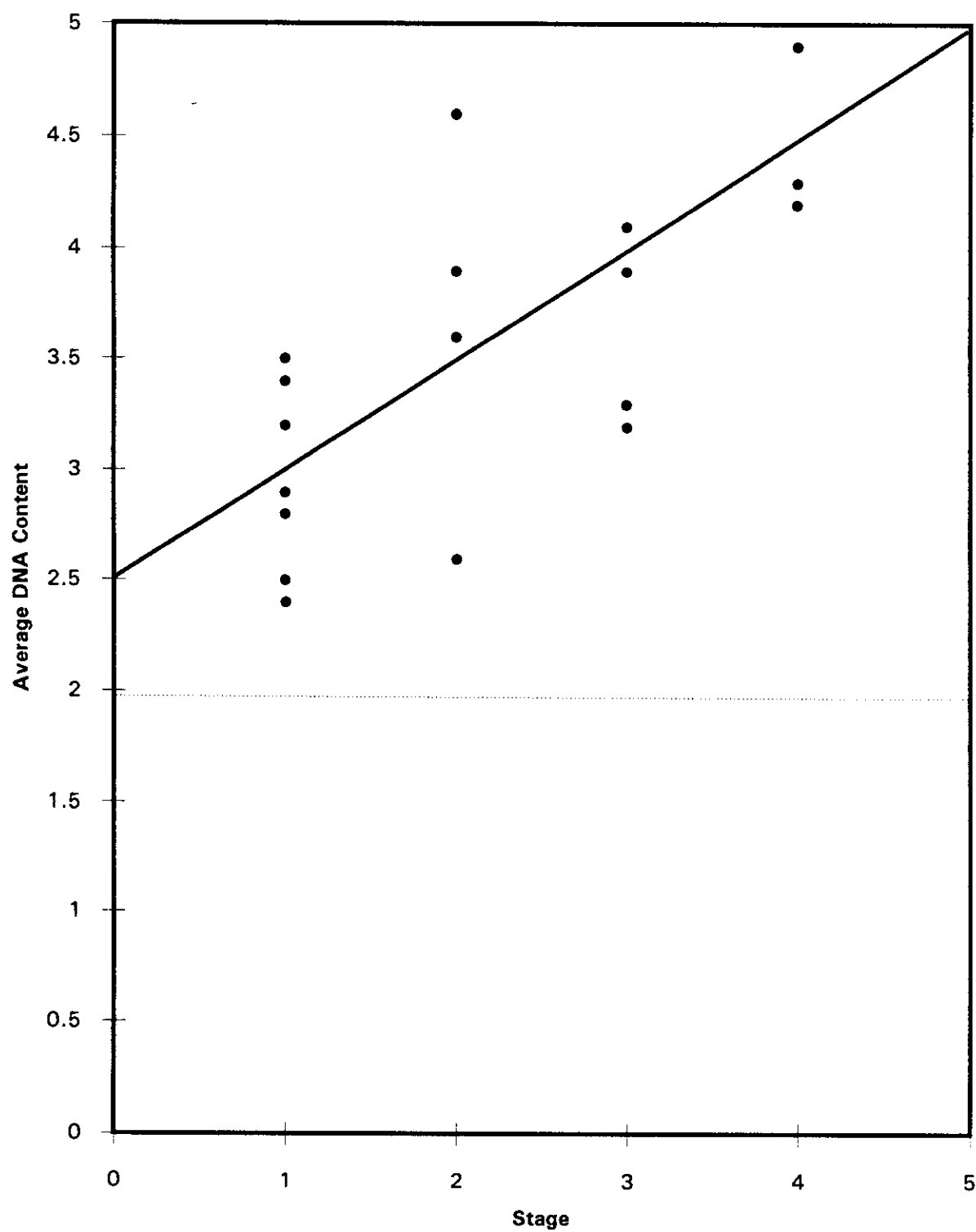
* Correlation between the type of histogram and tumor stage.

Comparing the type of histograms in relation to every stage, table (15) it was found that, the higher the cervical carcinoma stage, the more advanced the type of DNA histogram. Statistically, there is a positive correlation between the type of DNA histogram and the tumor stage (r = 0.61) and this correlation is of high significance (p= 0.01). Fig. (47).

Histograms of different tumor stages :-

(1) Stage I tumors:

Stage I group showed a histogram characterized by a single distinctly peak in the diploid region (2c) of normal control. Only small percentage of cells showed a little deviation than normal value. The percentage of cells above 4c was less than 10% and there is no cells above 6c. Thus stage I cervical carcinoma showed type I histogram. Fig.(48)

(2) Stage II cases.

This group of cases showed a histogram with most of the cells were below 4c but the percentage of cells over 4c are more than 10%

Also some cells were over 6c but they did not surpassed 10%. This histogram could be considered as type II Fig.(49)
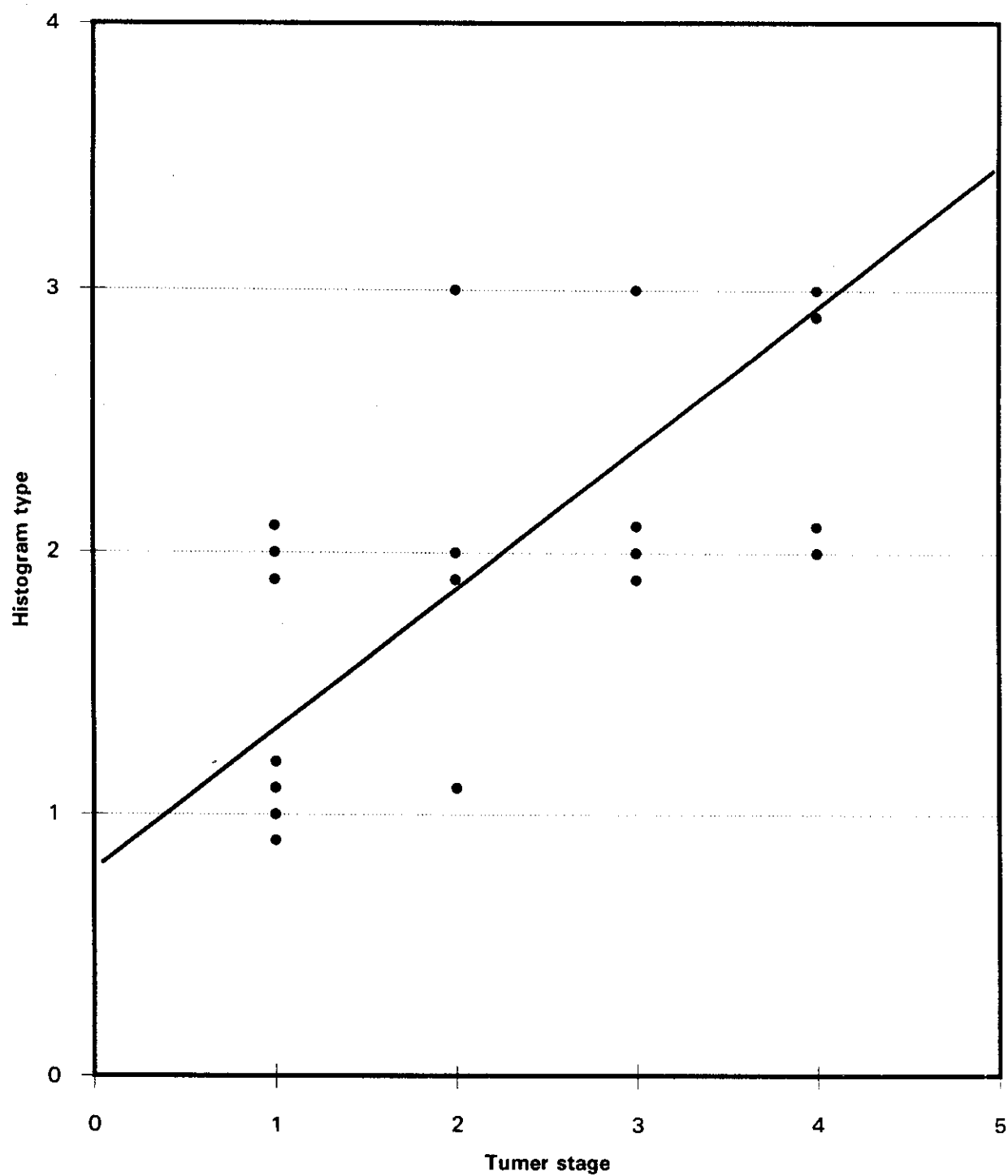
Fig. 47 : Correlation between histogram type and tumer stage

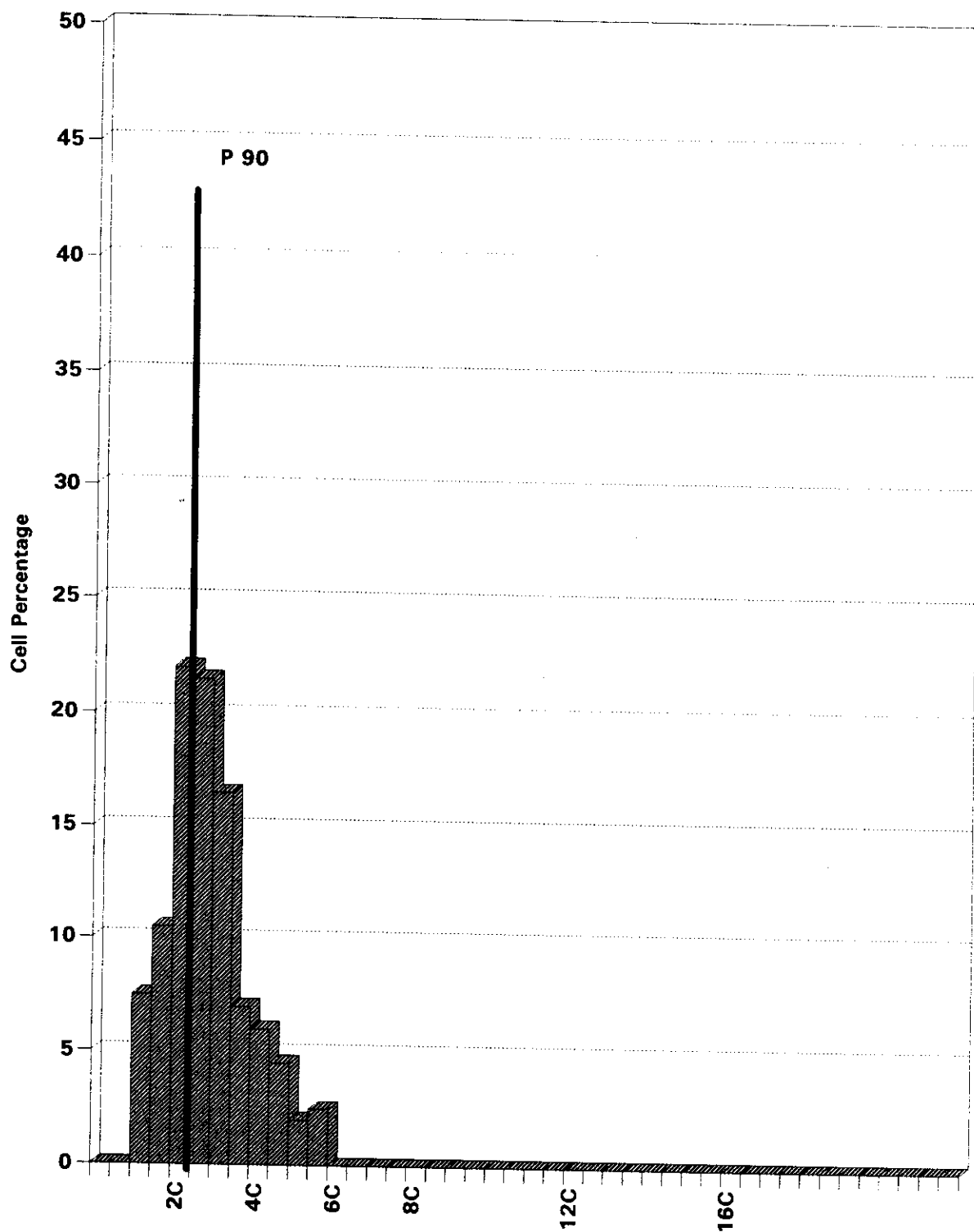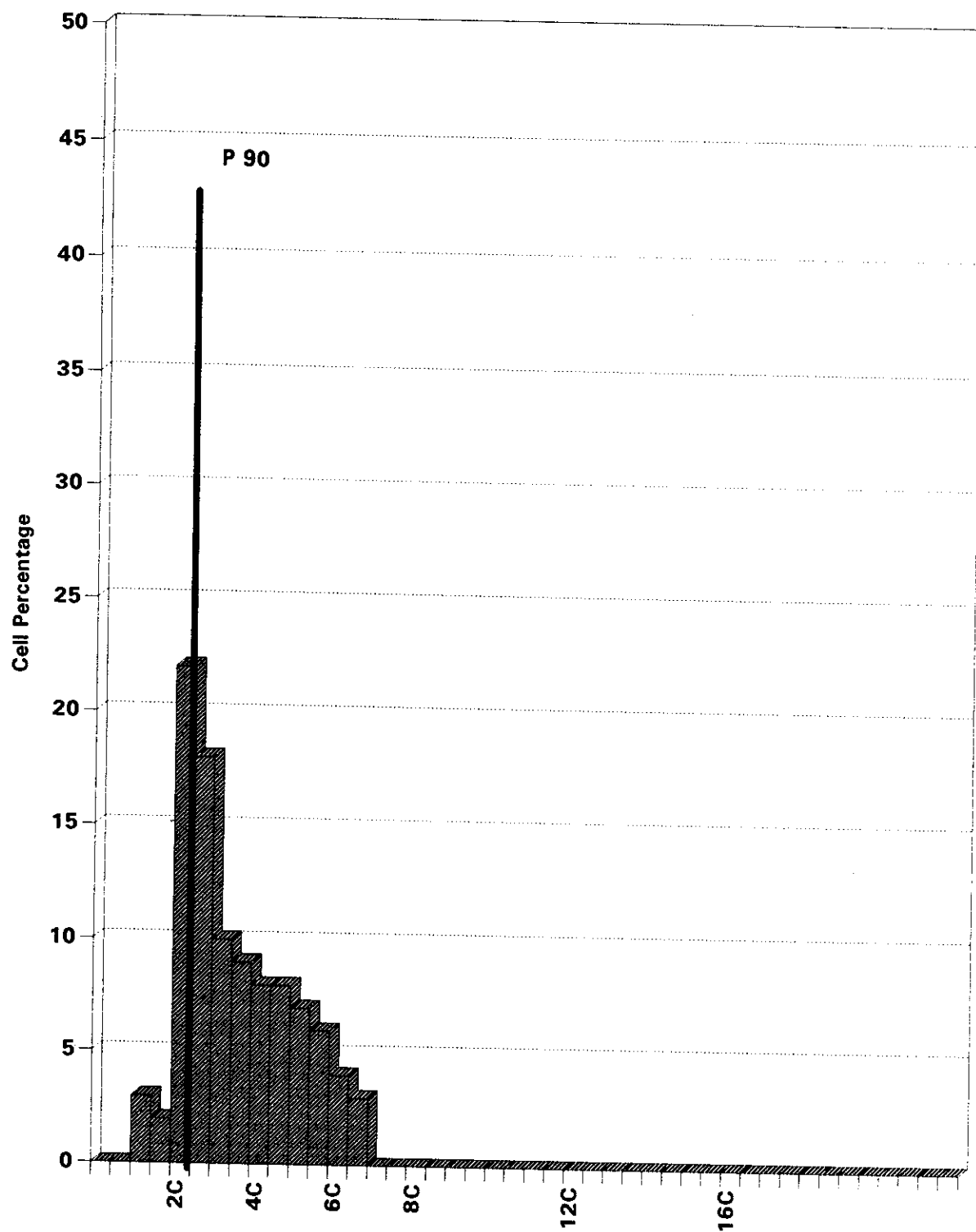**Fig. 48   : DNA Distribution (Stage 1)**

Fig. 49 : DNA Distribution (Stage 2)

(3) Stage III cases:

The histogram of cervical carcinoma stage 3 is more or less similar to that of the stage II, but with slight shift of the modal DNA value towards the 4c. The proportion of the cells surpassed 10% (about 20%) but those over 6c did not surpass 10% of cells. In spite of the large number of cells above 4c, stage III group presents also type II histogram. Fig.(50).

(4) Stage IV cases:

Stage IV cells showed a broadly scattering histogram and it was characterized by marked aneuploidy pattern and the individual DNA contents were ranged from levels near 2c up to value more than 12c with most DNA level over 4c. The proportion of cells over 6c surpassed 10% of cells (about 15%) . Thus stage IV cells presented type III histogram. Fig.(51).

Stage C	I	II	III	IV	row total
2- 3c	5 62.5 %	1 25%	0 0%	0 0%	6 30%
3-4c	3 37.5%	2 50%	3 75%	0 0%	8 40%
> 4c	0 0%	1 25%	1 25.0%	4 100%	6 30%
column Total	8 40%	4 20%	4 20%	4 20%	20 100%

Table (14) Relationship between tumor stage and average DNA content.

Stage Histogram type	I	II	III	Iv	row total
I	5 62.5 %	1 25%	0 0%	0 0%	6 30%
II	3 37.5%	2 50%	3 75%	2 50%	10 50%
III	0 0%	1 25%	1 25%	2 50%	4 20%
column Total	8 40%	4 20%	4 20%	4 20%	20 100%

Table (15) Relationship between tumor stage
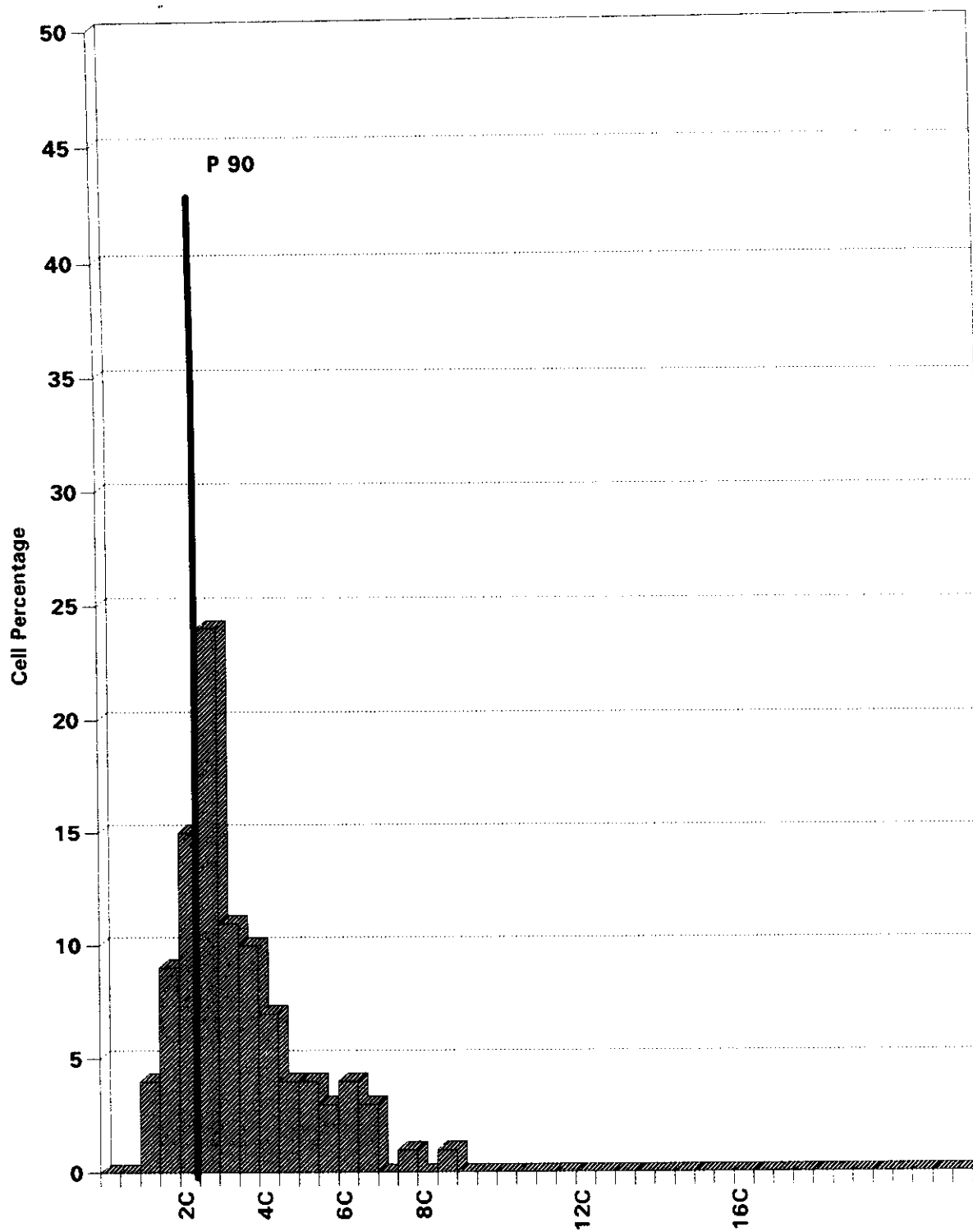*and type of histogram.*

Fig. 50   : DNA Distribution (Stage 3)

Fig. 51 : DNA Distribution (Stage 4)