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CHROMOSOME STUDIES ON CELL LINES AND CELL STRAINS OBTAINED FROM PRIMARY MONKEY KIDNEY CELL CULTURES

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In a previous paper [13] the establishment of cell lines and strains from pooled and individual monkey kidneys has been described. The present report gives an account on the changes observed in chromosome patterns during the development of lines and strains.

Material and methods

The lines and strains used were characterized previously [13]. Chromosomes were counted by the modified method of Rothfels and Siminovitch [11] as follows. Three or four day old tube-cultures were used. Fluid change was made with a medium, the initial medium for primary cultures and the propagating medium for lines and strains [13], containing colchicine in a 1 : 30 million dilution. After an additional incubation of 16 to 20 hours the medium was replaced with pre-warmed PBS [2] diluted 1 : 10 with distilled water and the cultures were reincubated for a further 30 minutes. This was followed by the fixation of cells for 10 minutes in a 1 : 3 mixture of acetic acid and ethanol. The fixative was poured off and the preparation was allowed to dry. After complete drying the cells were removed from the tube by the collodion membrane method [9] and stained under coverslip with 2 per cent neutral orcein in 50 per cent glacial acetic acid. Chromosomes of 50 random cells were counted on each slide.

Experimental

Chromosome patterns of primary monkey kidney cell cultures. A chromosome number of 42 was found [1, 10] to be the characteristic diploid number for the Rhesus monkey. Most of the cells in our primary cultures had 42 chromosomes (see Fig. 1 and Photo 1). There was, however, already in this early state a tendency towards irregular chromosome numbers, mostly smaller than the diploid value (hypodiploid), but haploids were also found (see Photo 2). Some cells exhibited hypotetraploid chromosome numbers.

Chromosome patterns of strains obtained from pooled monkey kidneys. Chromosome counts of lines obtained from pooled monkey kidneys were not determined, as this series of experiments served only for collecting preliminary information. The chromosome counts of strains No I/1/a, No II/1/a, No III/1 and No VIII/1/a were determined in their 46th, 44th, 44th and 37th passages, respectively.

Fig. 2 shows the histograms of these strains. In strain No III/1 there was an obvious peak at 84 (see Photo 3), i.e. at the tetraploid value of the Rhesus monkey. This strain was of fibroblastic character and was growing about half as fast as the others. Chromosome counts deviated more to the lower

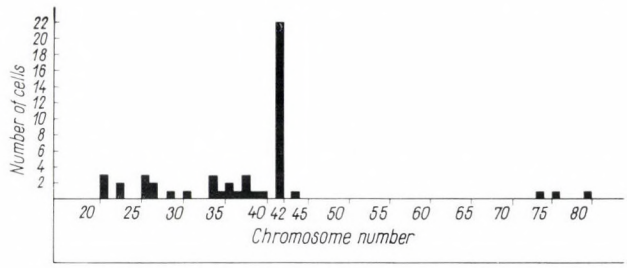

Fig. 1. Histogram of a five-day-old primary monkey kidney cell culture

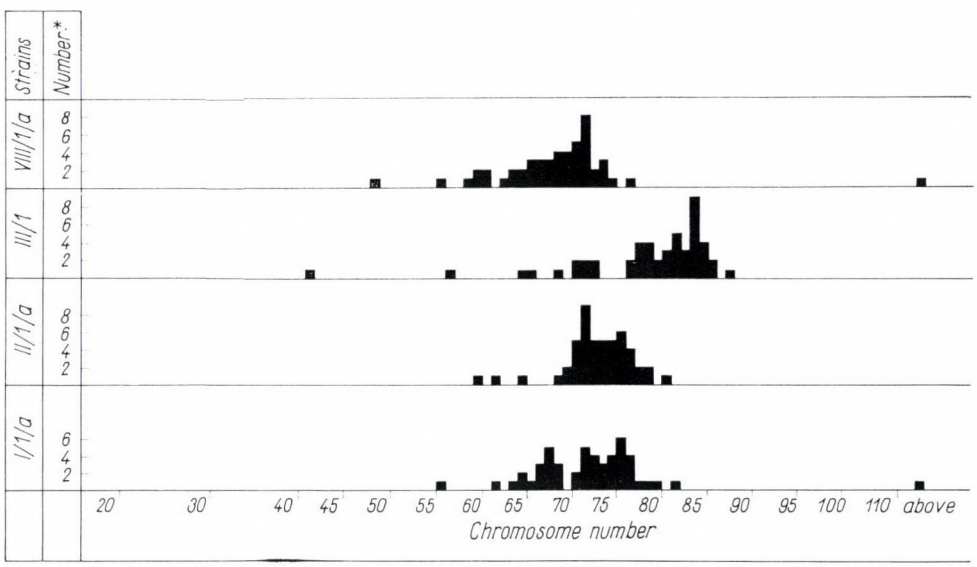

Fig. 2. Histograms of cell strains obtained from pooled monkey kidneys
* Number of cells exhibiting the appropriate chromosome number

than to the higher values. Histograms of strains No II/1/a and No VIII/1/a both had peaks at 72, thus they appeared to be of hypotetraploid character. In case of strain No VIII/1/a the deviation tended towards the lower, while with strain No II/1/a towards the higher values. The latter strain exhibited a secondary peak at 76. Strain No I/1/a gave no definite peak in the histogram, though three clear-cut maxima could be demonstrated at the values of 68, 72 and 76 (see Photo 4).

Chromosome patterns of lines and strains obtained from individual monkey kidneys. In the lines obtained from individual monkey kidneys, chromosome counts were determined between the 15th to 20th passages. Lines died out earlier were thus excluded from chromosome counting.

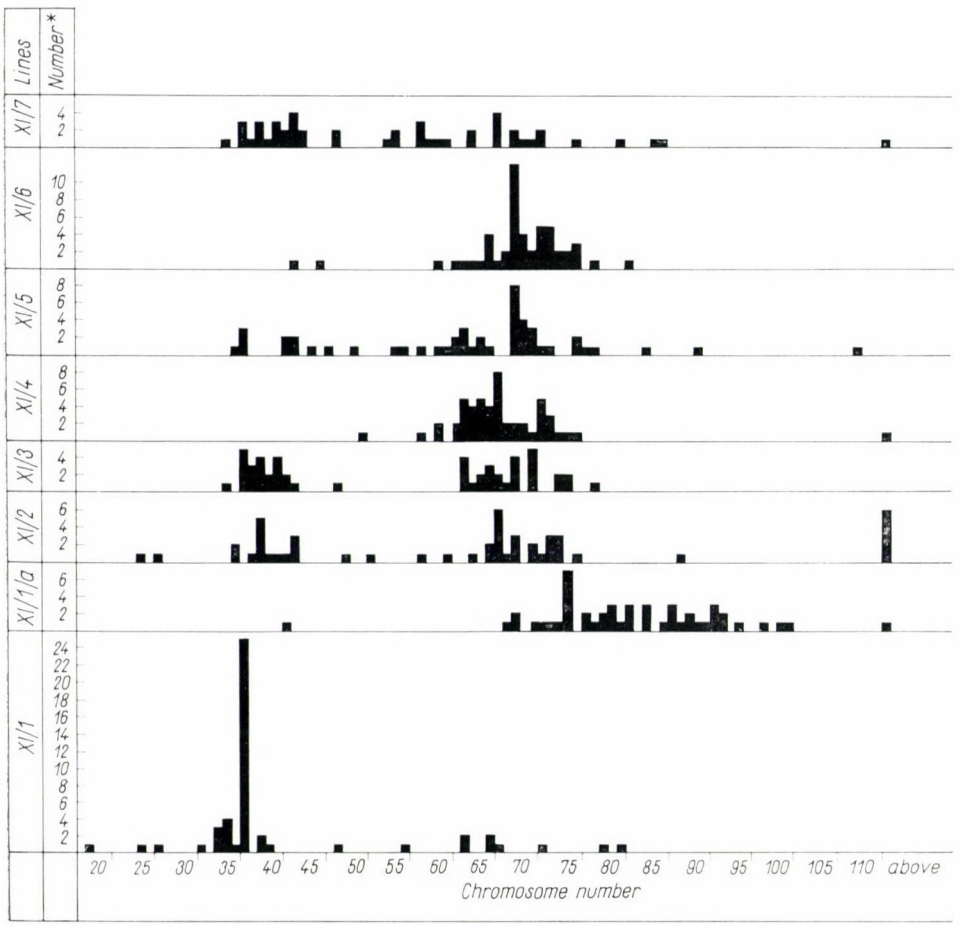

Fig. 3. Histograms of cell lines and strains obtained from monkey kidney No XI.
* Number of cells exhibiting the appropriate chromosome number

In the lines, except for line No XI/I obtained from monkey No XI, the main chromosome number rose to 66—70 (see Fig. 3 and Photo 5), thus these cells were of hypotetraploid character. Secondary peaks could be observed in the hypodiploid region and some highly polyploid cells were also found. In contrast, line No XI/1 had a high peak at 36, indicating a hypodiploid character. With this line, deviations were smaller and nearly symmetric as compared to the other six lines. Of the lines obtained from monkey No XI,

this was the only one giving later rise to a cell strain No XI/1/a. After ten passages following the establishment of strain No XI/1/a, its chromosome count was re-determined. The histogram obtained exhibited a peak at 74, and a considerable deviation towards higher values, i.e. the chromosome count of strain had increased more than twofold as compared to the mother line.

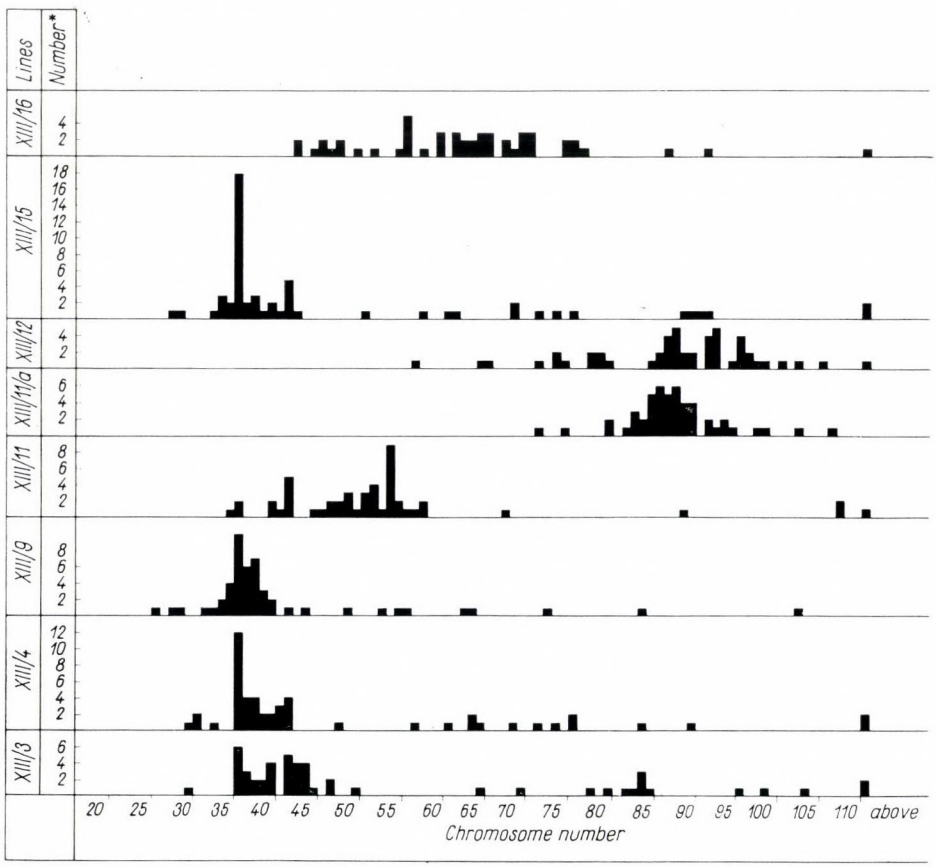

Fig. 4. Histograms of cell lines and strains obtained from monkey kidney No. XIII.
* Number of cells exhibiting the appropriate chromosome number

The histograms of the individual lines obtained from monkey No XIII showed great differences (see Fig. 4). Lines No XIII/3, No XIII/4, NoXIII/9 and No XIII/15 exhibited a peak at 36, i.e. the majority of the cells was of hypodiploid character (see Photo 6), with considerable deviation towards polyploids. Peaks at 54 and 56 were observed for lines No XIII/11 and No XIII/16 (see Photo 7), respectively. Deviations tended downward in the former, while upward in the latter. In the histogram of line No XIII/12 no maximum was found, while in the hypertetraploid region two peaks were present at 88

and 93, respectively (see Photos 8 and 9). All lines died out after different periods, except for lines No XIII/11 and No XIII/12. The latter has been propagated without difficulty up to now and the strain has remained apparently identical with its mother line. Line No XIII/11 showed, however, a labile period characterized by a poor growth of most of the cultures. After ten passages following that period the chromosome count was determined again.

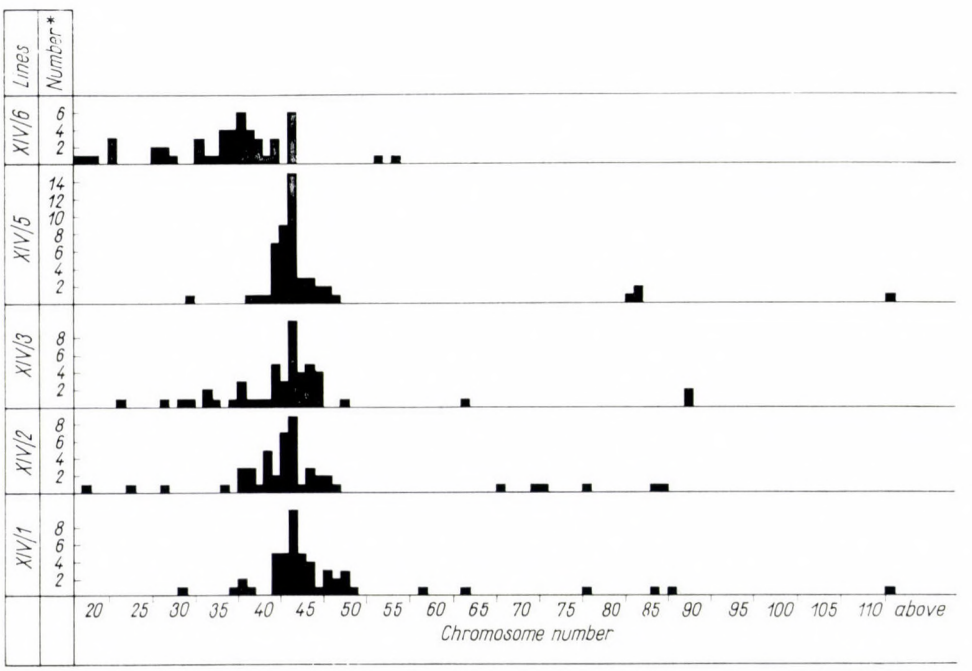

Fig. 5. Histograms of lines obtained from monkey kidney No XIV.
* Number of cells exhibiting the appropriate chromosome number

The increase of chromosome count was demonstrable also in this case; most values were between 85 and 90.

The peak chromosome counts in lines obtained from monkey No XIV remained at the original diploid number 42 (see Fig. 5), with a small deviation towards tetraploid values. A marked deviation towards the hypodiploid value 36 was demonstrable only in line No XIV/6. All these lines were of fibroblastic character and none of them gave rise to a cell strain.

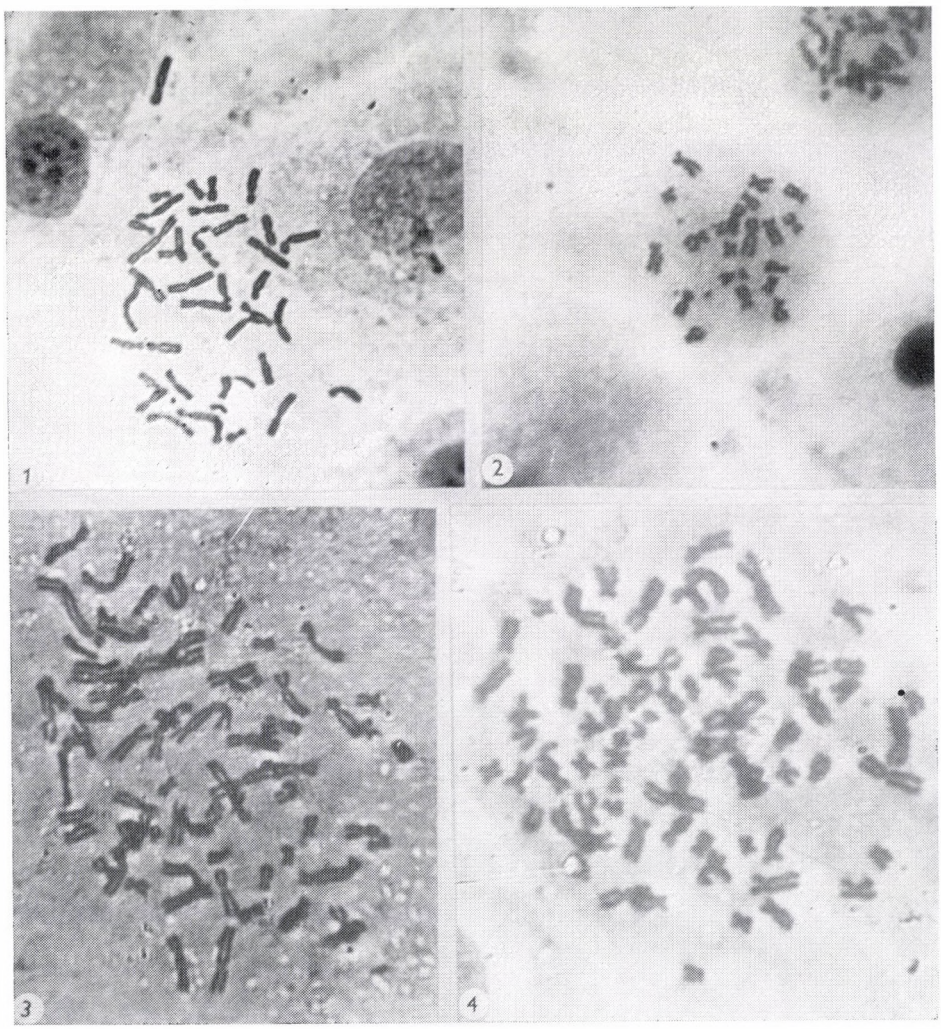

Photo 1. A diploid cell showing 42 chromosomes from a 5-day-old primary monkey kidney cell culture.

Photo 2. Haploid cell showing 21 chromosomes from a 5-day-old primary monkey kidney cell culture.

Photo 3. Tetraploid cell showing 84 chromosomes, from strain No III/1.

Photo 4. A cell showing 72 chromosomes from strain No II/1/a.

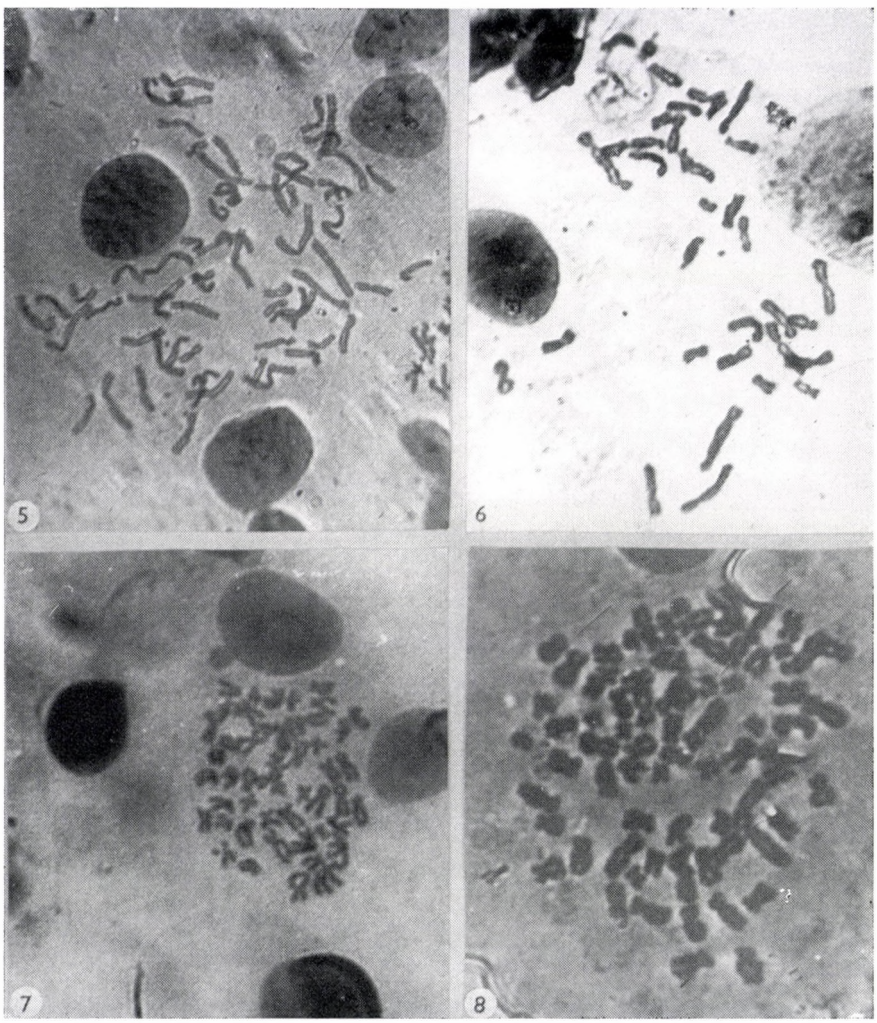

Photo 5. A cell showing 70 chromosomes from line No XI/3.
Photo 6. A cell showing 36 chromosomes from line No XIII/15.
Photo 7. A cell showing 54 chromosomes from line No XIII/11.
Photo 8. A cell showing 88 chromosomes from strain No XIII/12.

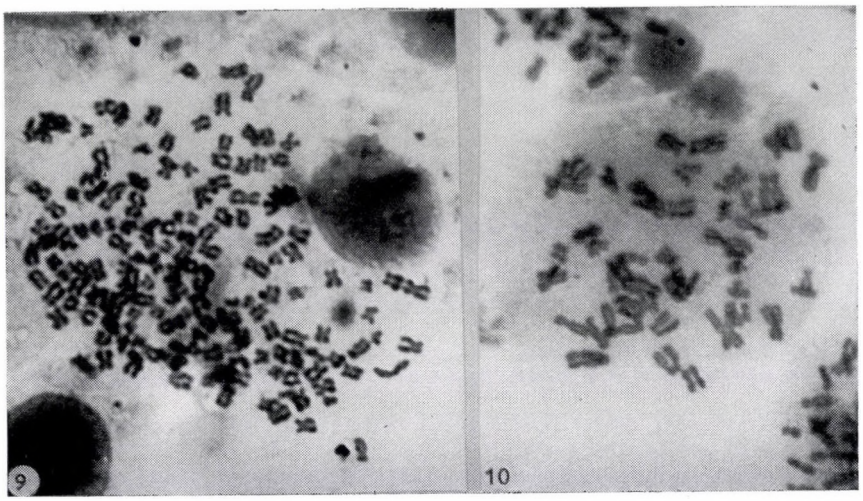

Photo 9. A highly polyploid cell containing about 160 chromosomes, from strain No XIII/12.
Photo 10. Endomitosis in a 5-day-old primary monkey kidney cell culture
Photos 2, 7, 9, 10 were taken by a Row. microphot B apparatus, ocular 10, objective 40, magnification ×240. Photo 1 was taken on a slide, ocular 5, objective HI 90, magnification ×450, phase contrast. Photos 3, 4, 5, 6, 8 were taken on slides, ocular 10, objective HI 90, magnification ×900, phase contrast

Discussion

The polyploidization of cells during prolonged cultivation *in vitro* was repeatedly demonstrated in both "transformed" and "non-transformed" strains [4, 5, 6, 7, 12, 15]. This process, however, does not seem to be the primary cause of the establishment of a strain or of "transformation". The fact that it is not responsible for "transformation" is evident as the "non-transformed" strains have also gone through this process [15]. The observation that cells of an established strain had been of diploid or rather hypodiploid character at the beginning and the chromosome number increased only later without any visible changes in the general morphology of the cells shows that polyploidization might have been a secondary process only [15].

In the experiments presented, striking changes in the chromosome pattern occurred during the development of lines and strains. As the majority of the lines derived from the same batch of monkey kidney showed similar changes or deviation tendencies, one was inclined to suppose individual differences between the animals. All six lines from monkey No XIV were of diploid character. The lines obtained from monkey No XI, except for line No XI/1, had increased chromosome counts (peaks between 66 and 70), with secondary peaks in the hypodiploid region. The greatest variations in chromosome number were found for lines obtained from monkey No XIII. Out of the

7 lines studied 4 had a decreased, 2 a moderately increased, and one a greatly increased chromosome number.

The survival of lines was influenced by the date of the first passage and by that of the establishment, as it has been demonstrated in a previous report [13]. The studies of the chromosome patterns indicate, however, that in addition to those factors the chromosome counts also had a role in determining survival. Thus the life period of lines obtained from monkey No XIII, characterized by hypodiploid chromosome numbers, was usually much shorter than that of lines obtained from the other two monkeys with diploid or hypotetraploid chromosome numbers. Of the lines derived from monkeys No XI and No XIV, No XI/2 and No XIV/1 exhibited the longest survival period, i.e. 53 and 55 weeks, respectively. On the contrary, with the lines derived from monkey No XIII, even the longest survival period did not exceed 38 weeks (line No XIII/3). The chromosome patterns did not seem to be related to the two points of time (date of the first passage and that of the establishment) found to influence the length of survival. Similarly, no relation could be detected between the changes in chromosome number and the establishment of cell strains. Lines from individual monkey kidneys, giving rise to strains on further cultivation, exhibited different histograms just like those which died out. The process of development of the strains appeared, however, to be related to the chromosome counts. Thus line No XIII/12 with a histogram in the hypertetraploid region could be propagated without any difficulty after its establishment. On line No XIII/11, having a main chromosome number in the hyperdiploid region with a peak at 54, showed a labile period during its cultivation. This period was characterized by a poor growth of a number of cultures. After the growth had improved the cells exhibited increased chromosome numbers (hypertetraploid). During cultivation of line No XI/1, with a histogram in the hypodiploid region with a peak at 36, a second lag phase occurred, when multiplication had almost stopped. After this period there was a high degree of polyploidy. All the strains derived from pooled monkey kidneys were of polyploid character, with a peak in the hypotetraploid region for strains No I/1/a, No II/1/a and No VIII/1/a, and at the tetraploid number 84 for strain No III/1.

From the data presented above it seems reasonable to differentiate cell lines from cell strains also on the basis of their chromosomal characteristics. The chromosome pattern of the lines presumably representing the first phase of adaptation to continuous growth *in vitro* appeared to be labile and exhibited great differences. The development of strains from lines seemed to be related to the above mentioned two critical points of time and was apparently independent of the actual chromosome count of the lines. Any line that had developed at other than the critical points of time died out independently of having had a hypodiploid (monkey No XIII) or polyploid (monkey No XI)

chromosome pattern. Nevertheless, at the end of the second phase of establishment, when strains had become stable, most of the cells exhibited polyploidy. It is, however, difficult to decide whether the second lag phase was necessary for the development of polyploidy or it served for the appropriate adaptation of the cells. Polyploid cells may be of different origin. Their presence in primary cultures at the 4th to 5th day of incubation has been demonstrated and the great variety of the ways of polyploidization was discussed by HSU [3] and LEVAN [5]. Atypical mitoses and endomitoses were found in our cultures, too (see Photo 10). It is not probable that the polyploid cells in the strains would be the same as in the primary cultures as e.g. strain No XI/1/a had been established during the 67th week of incubation. We do not know whether the original polyploid cells had survived until the establishment of the strains and, if so, why a period of 25 weeks had been needed for these cells to become dominant after the death of the mother line. Presumably, either the original polyploids had become adapted and selected, or the hypodiploids had undergone adaptation and selection and simultaneous polyploidization. Neither "transformation" nor mutation appeared to have taken place, as the development of lines and strains was not the result of a sudden change, while it seemed to be related to the time factor.

The lines obtained from monkey No XIV did not yield any strain. All these lines were of diploid and fibroblastic character. Attempts made by other authors to obtain human diploid strains have similarly failed; like in our experiments, the diploid, fibroblast-like cells were living for about one year only [8, 14]. An inadequacy of the media used was assumed to be responsible for the death of such cells. In our trials, the diploid lines died out gradually after different periods of survival, in spite of the fact that media prepared from the same ingredients and in the same way were always used. Apparently, it is not only the media that should be made responsible for the death of these cells. Under the experimental conditions applied the adaptability of fibroblast-like cells to continuous growth *in vitro* seems to be weaker than that of epithelial cells. Our experiments have pointed to the importance of the capacity to undergo polyploidization during the establishment of a strain. These cells presumably were unable to go through this process. Atypical mitoses were remarkably less in these fibroblast-like lines than in epitheloid ones. Our studies of this problem will be presented in another paper. The fact that fibroblast-like cells of strain No III/1 obtained from pooled monkey kidneys have their main chromosome counts at 84, the tetraploid value of the Rhesus monkey, seemed to support the above supposition. It seems to be of a certain interest, that these cells grow at a 50 per cent lower rate than the cells of the other strains. In this case, however, the interaction of cells from pooled monkey kidneys should not be excluded.

Summary

1. Studies on chromosome counts of lines and strains established in our laboratory from primary monkey kidney cell cultures have been presented.

2. In the lines a greater variety of the chromosome pattern was found than in strains. The individual differences in monkeys appeared to have a certain relation to the chromosome pattern found *in vitro*.

3. All strains were of hypotetraploid, tetraploid or hypertetraploid character.

4. The significance of polyploidization in the establishment of cell strains has been discussed.

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CHROMOSOMENUNTERSUCHUNGEN AN AUS PRIMÄREN AFFENNIEREN-ZELLKULTUREN ISOLIERTEN ZELLINIEN UND ZELLSTÄMMEN

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1. Die Chromosomenzahl von aus primären Affennieren-Zellkulturen isolierten Zelllinien und Zellstämmen wurde untersucht.

2. In den Zellinien ließen sich abwechslungsreichere Chromosomenbilder feststellen, als in den Stämmen. Die bei den Affen beobachteten individuellen Unterschiede äußerten sich im in vitro gefundenen Chromosomenbild.

3. Die Stämme wiesen einen hypotetraploiden, tetraploiden bzw. hypertetraploiden Charakter auf.

4. Die Bedeutung der Polyploidisation wird im Zusammenhang mit der Herausbildung der Stämme besprochen.

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ИССЛЕДОВАНИЕ ЧИСЛА ХРОМОСОМ В КЛЕТОЧНЫХ ЛИНИЯХ И ШТАММАХ, ПОЛУЧЕННЫХ ИЗ ПЕРВИЧНЫХ КЛЕТОЧНЫХ КУЛЬТУР ПОЧЕК ОБЕЗЬЯН

П. РУЗИЧКА

1. Сообщаются результаты исследований, проведенных с целью выяснения числа хромосом в клеточных линиях и штаммах, полученных в лаборатории из первичных клеточных культур почек обезьян.

2. В клеточных линиях были найдены более разнообразные картины хромосом, чем в штаммах. Индивидуальные отклонения обезьян проявлялись также в картинах хромосом, полученных **in vitro**.

3. Штаммы имели гипотетраплоидный, тетраплоидный и гипертетраплоидный характер.

4. Обсуждалось значение полиплоидизации в связи с образованием штаммов.