Chromosome Variation in Cell Populations Derived from Pig Kidney*

FRANK H. RUDDLE†

WITH THE TECHNICAL ASSISTANCE OF MRS. SHULAMIT KARBY

(Department of Zoology, University of California, Berkeley, Calif.)

SUMMARY

A stable line of pig cells (PK) which has been in cultivation for 5 years has been used to study chromosomal variation. This cell line, together with its variously clonally derived sublines, has continued to possess a diploid component. Tetraploid and heteroploid components were also present.

X-ray treatment of the PK cells produced a number of reciprocal translocations, resulting in chromosomes with unique morphology. Such chromosomes were extremely stable and conferred marker properties on populations which contained them.

In the study of in vitro clones over a period of a year, it was found that while such populations are initially quite uniform, variation tends to increase with respect to distribution of chromosome number and different ploidy groups. In some instances, population heterogeneity could be attributed to the cloning procedure itself.

A number of investigations on chromosome patterns in established cell cultures have shown that significant departure from the normal karyotype can occur in terms of aneuploidy and heteroploidy, and that such populations are commonly mosaic in nature, being composed of two or more stemlines (9, 17). The nature and evolution of karyotypic alterations have been outlined in several reports (8, 11), and there have also been reports on the long-term cultivation of cells with little or no karyotypic change (15, 19). In the present experiments chromosome patterns have been used to investigate two aspects of population dynamics. One of these is the question of homogeneity and stability in populations which have been derived from single cells. A second is concerned with chromosomal markers: the experimental production of individual chromosomes with a unique morphology, which can be used to identify a given cell strain and follow its further evolution. In this way, chromosomally marked lines can be combined under various conditions and the fate of the populations followed. Several studies on spontaneously arising marker chromosomes have shown this to be an operational approach (7, 12).

The work reported here describes the creation of six cell populations with chromosome markers, all derived from a stable line initiated from pig kidney. The stability of these populations is examined on the basis of several criteria. The experiments and observations aim to provide information on the inherent variability of cell populations, how variation may be controlled, the methods of introducing marker chromosomes into karyotypes, and information on their stability.

MATERIALS AND METHODS

Origin and culture of cell lines.—The pig kidney cell strain used in these studies was initiated by Dr. E. Stice of Cutter Laboratories, Berkeley, California, in November, 1955. Cultures were maintained on a nutrient medium composed of 50 per cent medium 199 (18), 40-50 per cent Hanks or Earle saline, 5-10 per cent Lamb serum, penicillin (100 units/ml), dihydrostreptomycin (100 μg/ml), and phenol red (0.002 per cent level).

* This investigation was supported by a predoctoral fellowship, CF-8991-C1, from the National Cancer Institute; and by Grant RG-5085, Division of Research Grants, U.S. Public Health Service.

† Present Address: Department of Zoology, Yale University, New Haven, Conn.

Received for publication January 24, 1961.
Cell populations were cultivated in 8-oz. medicine bottles directly on glass and were removed from the substrate by means of 0.1 per cent trypsin and 0.1 per cent versene in Puck's solution A (14).

Cloning.—Cloning was performed in most instances according to the method of Puck (15). Cells were plated out in concentrations of 100 cells per 35-mm. diameter petri dish. Colonies were easily picked by isolating them with steel rings and then detaching the cells with trypsin-versene.

Chromosome analysis.—Cells were stained for chromosome analysis when they neared the end of the log phase of growth. Mitosis was arrested by colchicine, which was applied so that the final concentration was $1 \times 10^{-7}$ M. Colchicine was permitted to act on the cells for 6–8 hours in most instances. Cells were loosened from the glass by means of hypotonic trypsin-versene, which combined hypotonic treatment with trypsinization. Hypotonic trypsin-versene contained only 20 per cent of the NaCl salt usually present in Puck's solution A, and the trypsin concentration was reduced to 0.01 per cent in order to lessen cell fragility. Following 5–10 minutes of treatment with hypotonic trypsin-versene, the cells were gently pipetted until they were well separated and evenly suspended. Next, 0.5 ml. of hypotonic serum (3 parts serum to 7 parts distilled water) was added to the cell suspension to reduce clumping. Cells were then centrifuged at 500–800 r.p.m. for 5 minutes in Wassermann tubes. Wassermann tubes were useful, because the cells spread over the broadly curved bottom surface and thus more readily mixed into suspension without clumping when the stain was added. Supernatant fluid was drained away, and then 0.25–0.5 ml. of 2 per cent Gurr's natural orcin in 50 per cent acetic acid was added, and the cells were brought into suspension by gentle pipetting with a Pasteur pipette. After 5 minutes' staining, about 0.05 ml. of 1 N HCl was mixed into the cell suspension. Hydrochloric acid served to decrease precipitation of stain, sharpen differentiation, and, by toughening the cells, reduce breakage. Several drops of cell stain fixative were placed on siliconized slides and then squashed under No. 1 Dow Corning, 22-mm. square cover glasses. Squashing was accomplished by means of thumb pressure and also by means of a cell press of our own manufacture similar to one used by Beatty (1). Pressures of 80–100 pounds per square inch for periods of several seconds gave good results. The edges of the cover glasses were sealed with Kronig's cement. Such preparations were relatively durable and retained good cytological properties for as long as a year when stored at 4°C.

Recording observations.—Chromosome counts were usually made with the use of an ocular grid and handcounter. The ocular grid was not used when chromosomes were of low number and well spread. Two independent counts were made, and each value was recorded. Both values were plotted in distributions of chromosome numbers. Counting error has been discussed elsewhere (17).

Morphological studies on chromosomes were done by two means. In one, the chromosomes were drawn with the use of the camera lucida. In the other, the chromosome array was photographed on 4 × 5 film and then enlarged. From such pictures, individual chromosomes were cut out and then arranged as idiograms. Photo-idiograms have the advantage of greater fidelity in comparison with those obtained with the camera lucida. For notebook records, photographs were made with a Land Polaroid Camera.

Estimates of the percentage of diploid versus polyploid cells in metaphase were made by scanning a colchicine-squash preparation and then scoring all diploid and high ploid cells encountered regardless of their suitability for counting. This method gave a more realistic view of cells in the diploid region, since the greater difficulty of counting cells at higher ploidy level did not tend to exclude some of these cells from the data.

X-radiation.—Chromosome rearrangements were induced by means of x-radiation. A 250-kv. x-ray machine was used to irradiate the cultures, and the beam was filtered by means of 1.0 mm. Al and 0.5 mm. Cu filters. Distance to the cell sheet was 16 cm. from the bottom of a 15 cm. × 20 cm. cone. Delivery rates were measured by means of a Radocon monitoring device placed at the same height and beside the cell sheet. All exposures were made at 59 r/min. Cells were irradiated in 3-oz. prescription bottles, the cell sheet being turned upward so that radiation traversed one layer of glass before striking the target. Medium was removed during irradiation and replaced after 3 hours, and a 50 per cent fluid change was made at that time.

Preservation of cells by freezing.—A number of cell strains studied were preserved by freezing to obviate their accidental loss and also to provide samples of these strains from specific times in their cultural histories. Cells were preserved by low temperature storage in 10 per cent glycerol in nutrient medium. A Revco mechanical freezer operating at $-90°C$. was used for storage.

Clone terminology.—The terminology used to describe the clone populations was as follows: The
original uncloned population of E. Stice which had been in continuous cultivation was termed PK-1. Clones of PK-1 were termed PK 1-1, 1-2, etc., while clones of PK 1-2 were termed PK 1-2-1, 1-2-2, etc. Thus in this nomenclature the first number place indicates the parental population. The second indicates a particular clone derived from the parent. The third determines a particular clone which arose from a population in the first generation of clones and so on.

RESULTS

Outline of Experimental Work

PK-1 was subjected to chromosome analysis, and several clone populations were established by means of single cell isolation. PK 1-2 was exposed to x-radiation, and from the survivors 68 clone populations were established; of these, 48 were screened for unusual rearrangements of the large chromosomes. Fourteen clones having good chromosome marker properties were saved and then recloned to ensure populations which were derived from single cells. In almost all instances, five subclones were prepared. Those sublines best fulfilling criteria of homogeneity, stability, and good growth were used to continue the stocks. Six sublines which seemed representative of the original fourteen were selected and then subjected to detailed study. These lines were cloned a third time in a number of instances. A schematic outline of the descent of clones is given in Chart 1.

Karyotype of the pig.—The karyotype of the Hampshire pig was obtained by the study of 1-week-old cell cultures of kidney tissue. It was found that the 2n number was 38, and that the chromosomes could be divided into eight morphologically distinct groups. The criteria used to distinguish different chromosomes morphologically was that originated by Levan (10). Examples of the morphological groups are given in Figure 1, and a short description of these groups is given below.

Group:

I (1) Longest submetacentric (SM) chromosomes.
II (1) Medium-small SM chromosome with a long, constricted area.
III (2) Longest subacrocentric (SA) chromosomes.
IV (6) Large-medium, to medium-small metacentric (M) and SM chromosomes.

The X chromosome also belongs to this group, bringing the diploid number to thirteen in the female.

V (2) Small M chromosomes.
VI (1) Longest acrocentric (A) chromosome.
VII (2) Medium A chromosomes.
VIII (3) Small A chromosomes.

The Y chromosome is a very small, slightly SM chromosome.

PK 1.—The uncloned parental population had 63 per cent of the cells at the diploid level, with a numerical variation about a mode of 38 chromosomes (Chart 2). A small component was present at the tetraploid level and a larger component at a hypotetraploid level, with a mode of 62–63 chromosomes. The karyotype was similar to that of the normal pig, there being 2 G-I, 2 G-VI, and 4 G-VII chromosomes. All other groups were represented, except the Y chromosome. The cells grew in an epitheloid fashion.

PK 1-1, 1-2, 1-3.—Three clone populations were obtained from PK 1. One (PK 1-1) was heteroploid, whereas the other two (PK 1-2, 1-3) were composed mainly of near diploid cells. The heteroploid clone PK 1-1 had a principal mode in the 60–64 region and clearly stemmed from the heteroploid component of PK 1 (Chart 2). During later months a diploid level component appeared in PK 1-1. Two explanations for this are possible, one being that a diploid component arose from the heteroploid by chromosome loss, the other being a contamination from an outside source. It was not possible to distinguish clearly between these two possibilities.

PK 1-2 and 1-3 both contained low ploid components whose modes were 38 and 39, respectively. PK 1-3 contained a heteroploid component (62–63) 1 month after cloning, which demonstrates the rapidity with which high ploid components can arise. The ploidy ratio of PK 1-3 was 90 per cent, and the distribution of chromosome numbers showed that the majority of the high ploid cells existed at the heteroploid level. The chromosome morphology of PK 1-3 was similar to that of PK 1.

PK 1-2 was studied in more detail, since it was this line which was used in the production of marker lines by means of x-ray treatment. Chromosome number distributions were made in July and October of 1958 (Chart 2). These distributions showed that the low ploid component

1 In other articles PK-1 has been termed PK-2n, which conformed to the original designation of Stice. The term PK-1 has been used here for simplicity. Other synonyms for the designations given to clones in this paper are already in the literature. These synonyms are given in parentheses in Chart 1.

3 Numbers in parentheses refer to the haploid number of chromosomes in the Hampshire karyotype.

4 Ploidy ratio refers to the percentage of diploid level cells.
varied only slightly about a mode of 38. The ploidy ratio for the July sample was 94% per cent diploid, while by October the frequency of diploid level (low ploidy) cells had dropped to 78 per cent. The increase of high ploidy cells went together with appearance of a heteroploid component in the October population. It was also of interest that the tetraploid component diminished, and its mode also shifted downward from 76 to 75 during the 3-month interval.

The chromosome morphology of PK 1-2 was relatively constant. Idiograms are shown in Figure 2. A large SM chromosome is shown in one of the idiograms of PK 1-2 (Fig. 2B). This resulted from the centric fusion of a G-VI and a G-VII chromosome. About 1.0 per cent of the population contained this pattern.

A comparison of PK 1-2 with the normal pig karyotype indicates the following changes which have occurred at some time during its 3 years of continuous cultivation. In PK 1-2, two large, constricted chromosomes are present, whereas these are absent in the normal karyotype. These may be

The term centric fusion is used in an operational sense to describe the joining together of two A chromosomes at their proximal ends with the resultant formation of an M chromosome. The term is synonymous with centric or Robertsonian interaction. The terminology is in no way meant to suggest the mechanism of the process.

**Chart 1.**—Chart showing the genealogical relationships between the parental uncloned and descendant cloned populations. The symbols in parentheses are synonymous with those symbols above them. The older symbols in parentheses have already appeared in the literature, and for that reason they are included here.

**Chart 2.**—Chromosome number distributions. A: PK 1; B: PK 1-2 (PK 1-2 (October of the same year)).
chromosomes which are grouped in G-VI in the normal karyograms but which have a more constricted appearance in PK 1-2. This might represent a difference in morphological behavior rather than a physical rearrangement. There is also a lack of one small G-III chromosome and the gain of one G-VIII chromosome. These changes can be most easily accounted for by nondisjunction. It can be seen that, whereas PK 1-2 has a 2n number of 58, its morphology is not strictly diploid but quasi-diploid. There was no evidence of translocation other than centric fusion in the PK 1-2 stemline.

X-Radiation of PK 1-2.—Having obtained information as to the homogeneity and chromosome constitution of the low ploidy stemline of PK 1-2, we introduced chromosomal variants into the population by means of x-ray treatment.

Replicate cultures of PK 1-2 cells were x-radiated at 300 r, 500 r, and 800 r. The populations showed the effects of irradiation within 24 hours by heightened sloughing of cells, sloughing being pronounced in the 800 r cultures. At the third transfer (second for the 800 r series) after a period of 33 days, the populations began to show good recovery, and yields of a normal magnitude were obtained. Just prior to this transfer, it could be seen that heteromorphic cell sheets were forming. This was especially evident in the 800-r cultures, where a number of focal areas of growth were developing, having obvious morphological differences. The colonies differed in terms of size, sharpness of outline, tendency for the cells to grow in multilayers, to form cords and balls of cells. The individual cells also varied in terms of size, shape, granularity, cohesiveness, etc. The over-all appearance was that of distinct variant populations growing up from recovering or initially undamaged individual cells. The fact that many variants were present in the population was later borne out by an examination of the chromosomes of these cells. Many chromosomal rearrangements were seen in cells from these populations, some of which were isolated and then cultivated as homogeneous populations.

There was no significant difference between the 300-r, 500-r, and 800-r categories, but this was probably due to the small number of clones in the 300-r and 500-r series. Twenty cell lines grew so poorly or gave such equivocal results that they were not scored. Of the 48 remaining clone populations, 22 (46 per cent) showed a translocation, usually explained in terms of a two-hit event. However, about half of these (ten out of 22) were of a centric fusion type. When Robertsonian alterations are excluded, major alterations explainable in terms of reciprocal translocations were found in 25 per cent of the clones studied. It should be stressed that no reliable estimate of the incidence of translocation produced by x-ray can be made from these data, because of the possibility of differential growth of survivors between the time of x-ray treatment and cloning.

PK 1-2-20.—Idiograms of PK 1-2-20-1 are shown in Figure 3. The marker chromosomes were a large SM chromosome of the G-1 type with a shortened long arm and an A chromosome intermediate in length between the G-VI and G-VII chromosomes. Markers were presumed to have arisen through a translocation between G-I and G-VII chromosomes. PK 1-2-20, the parental line from which PK 1-2-20-1 was cloned, had a ploidy ratio of 98 per cent prior to cloning and had a homogeneous appearance. Of the four clones which stemmed from this population, PK 1-2-20-1 was selected to continue in cultivation because of its high degree of homogeneity and good growth properties. PK 1-2-20-4 and 1-2-20-5 possessed an M chromosome which was probably derived from two G-VII type chromosomes by centric fusion. Interestingly, this chromosome was absent in the sister clones PK 1-2-20-1 and 1-2-20-3. Clone PK 1-2-20-5 showed a significant increase in high ploid cells compared with its sister clones, as well as with the parental clone PK 1-2-20.

PK 1-2-23.—Idiograms of PK 1-2-23-2 are shown in Figure 4. The marker was a long SA chromosome and probably arose through a translocation between a G-VI and a G-III chromosome. PK 1-2-23, the parent of PK 1-2-23-2, was homogeneous at the time of cloning, having a ploidy ratio of 96 per cent. Second-generation clones PK 1-2-23-1, -2, and -5 showed the parental pattern. Clone PK 1-2-23-3 was observed in early cultivation to exhibit areas of large, clear spindle-laid cells. Later analysis indicated a ploidy ratio of 88 per cent, and chromosome counts showed the presence of a slightly subhexaploid component having a modal value of 97. No cells of this type were observed in the parental population, and the component appears to have arisen during the cloning operation. Cells of this type possessed three long SA and SM chromosomes.

PK 1-2-20.—Idiograms of PK 1-2-20-3 are shown in Figure 5. The markers were an SM chromosome of the G-I type, which frequently displayed a secondary constriction in the long arm, and a minute chromosome of the A type. Markers probably arose through a translocation between a G-VII and an SM chromosome of the G-IV type. The minute fragment may represent the short arm of the A chromosome, and the
secondary constriction of the SM marker is perhaps due to the translocation of a portion of the centromeric region. The minute chromosome has been consistently observed in PK 1-2-26-3 for over a year. This signifies the genetic importance of this small fragment. If the minute represents the short arm of an A chromosome, this would indicate that the proximal arm in such chromosomes has a genetic function as well as its stated role as a centromere stabilizer (18). PK 1-2-26 was very homogeneous, with a low ploid component comprising 97 per cent of the population. Two clones derived from PK 1-2-26 had low ploid components: PK 1-2-26-2 with a ploidy ratio of 71 per cent, and PK 1-2-26-3 with a ploidy ratio of 87 per cent. Clone PK 1-2-26-1 lacked low ploid cells altogether.

PK 1-2-43-2 exhibited the unique possession of an interphase marker condition. This condition takes the form of a nuclear projection or bleb. It was believed that the nuclear projection was caused by the marker chromosome.

PK 1-2-51.—The marker chromosome was of the M type and had arisen by the centric fusion of two A chromosomes from G-VII.

PK 1-2-65.—Idiograms of PK 1-2-65-1 are shown in Figure 7. The marker chromosome was of the M type and had arisen by the centric fusion of two A chromosomes from G-VII. PK 1-2-65 was homogeneous with a predominantly low ploid contribution accounting for 97 per cent of the population. PK 1-2-65-1, -2, and -3 had ploidy ratios of 97 per cent. PK 1-2-65-4 had a ploidy ratio of 49 per cent, whereas PK 1-2-65-5 was 86 per cent. There was good evidence that clones which showed a decreased ploidy ratio also suffered the loss of one chromosome in that component. Probably this loss was related to the low frequency of low ploid cells in these populations. It was also noted that PK 1-2-65-1 cells had acquired an SM chromosome which probably arose through translocation.

Chromosome changes with time.—Chart 3 shows how ploidy ratio declined over a period of time in six clone derived populations. It can also be seen that, whereas the tendency for ploidy ratio to decrease was general, certain clones such as PK 1-2-65-2 and 1-2-51-1 remained at a high level with respect to percentage of near diploid cells. It was of interest that the cells in these populations underwent pronounced morphological change in terms of cell shape, and perhaps this fact was related to continued stability of the ploidy ratio.

In some instances, there was observed a distinct drop in frequency of low ploid elements. This was true of PK 1-2-26-3 which, after 5 months of
cultivation, dropped from a ploidy ratio of 87 per cent to one of 60 per cent. However, a third-generation clone, selected on the basis of a high ploidy ratio value, continued at a high level for 6 months.

Distribution of chromosome number was particularly stable through 5 months of cultivation, there being little variation about the low ploid modes (Chart 4). After 13 months cell lines PK 1-2-20-1, 1-2-23-2, 1-2-26-3, and 1-2-43-2 showed a tendency for the low ploid component to develop a mode in the low forties. It seems probable that the hyperdiploid cells originated from the diploid by an acquisition of chromosomes following nondisjunction. Also, an increased spread in the distribution of chromosome number was observed in the high ploid cells.

Chromosome morphology with respect to the large chromosomes and particularly the marker chromosomes has proved to be exceptionally stable. In more than over a year of continuous cultivation and in some instances through three successive cloning procedures a marker chromosome has never been lost or modified. This is also true of marker populations which have been cycled repeatedly through metabolic inhibitors such as diaminopurine and azaguanine with concomitant selection of drug-resistant strains (6). The stability of marker chromosomes is certainly a matter of both practical and theoretical interest.

Table 1 shows a degree of variability in the chromosomal condition of individual karyotypes. Such data confirm the reality of slight numerical variation which is always found in such populations and indicates that such variation exists, apart from counting error. A similar conclusion was reached by Court Brown et al. (4) on studies of short-term blood cultures. The data also show that, while chromosome number between cells may be the same, the number of certain kinds of chromosomes may vary. This is comparable to the "quasi-diploid" situation described for cultures of Chinese hamster cells (5). Such variations can be most readily attributed to nondisjunction.

The occurrence of nondisjunction, and the

---

DISCUSSION

Analysis of marker line idiograms indicated that the over-all chromosome patterns of individual cells within a line were predominantly of a single kind, but that variants from the predominant pattern also existed (Table 1). The data suggest that the predominant type represented the stem-line, whereas deviating types represented cells having short life expectancy or secondary stem-lines at the low ploid level. PK 1-2-23-2 showed two major patterns: one in which there were 12 G-IV and 8 G-VII chromosomes (Figure 4A), the other in which there were 13 G-IV and 7 G-VIII chromosomes (Figure 4B). This strongly suggests that two stemlines were present in this population.

Table 1 shows a degree of variability in the chromosomal condition of individual karyotypes. Such data confirm the reality of slight numerical variation which is always found in such populations and indicates that such variation exists, apart from counting error. A similar conclusion was reached by Court Brown et al. (4) on studies of short-term blood cultures. The data also show that, while chromosome number between cells may be the same, the number of certain kinds of chromosomes may vary. This is comparable to the "quasi-diploid" situation described for cultures of Chinese hamster cells (5). Such variations can be most readily attributed to nondisjunction.

The occurrence of nondisjunction, and the

---

**Chart 4.**—Chromosome number distributions. A: PK 1-2-20-1; B: PK 1-2-23-2; C: PK 1-2-26-3; D: PK 1-2-43-2; E: PK 1-2-51-1; F: PK 1-2-65-1.

---

Downloaded from cancerres.aacrjournals.org on October 3, 2017. © 1961 American Association for Cancer Research.
demonstrated survival of at least some cells resulting from such events, open up possibilities for a genetic analysis of cell populations. On a cytological level, it may be possible to utilize nondisjunction to relate specific enzymes or other gene products to specific chromosomes. Marker chromosomes may also have possibilities for genetic research. Marker lines can be employed to detect and study the possible fusion of diverse cells, an event which might contribute to a genetic analysis or organisms by means of their cultured cells.

Large, homogeneous populations of PK cells show good stability for relatively long periods of time with respect to a number of criteria. However, when such populations begin to exhibit increased heterogeneity, it may be necessary to resort to cloning in order to restore uniformity. The effectiveness of cloning in this respect has been illustrated above with clone PK 1-2-26-3. However, in the course of analyzing clones which had arisen from highly homogeneous parental populations, in some instances unexpected variation was found soon after cloning.

There are two explanations for variation between and within sister clones. One is that variant cells existed in the parental populations. Undoubtedly this accounts for the greater part of observed variation. This source of variation was clearly demonstrated when variants could be detected in the parental population and then grown in pure form as clone populations. Such variations

| TABLE 1 | VARIATION IN CHROMOSOMAL TYPES IN SIX CLONE-DERIVED CELL POPULATIONS |
| --- | --- | --- | --- | --- | --- | --- |
| Cells | Chromosome types | G-I | II | III | IV | V | VI | VII | VIII | Total with markers |
| PK 1-2-20-1: Cell 238 | 1 | 2 | 2 | 14 | 3 | 2 | 2 | 9 | 37 |
| " 252 | 1 | 2 | 2 | 14 | 3 | 2 | 2 | 9 | 37 |
| " 254 | 1 | 2 | 2 | 13 | 3 | 2 | 2 | 9 | 36 |
| " 246 | 1 | 2 | 2 | 14 | 3 | 2 | 2 | 9 | 37 |
| " 241 | 1 | 2 | 2 | 14 | 3 | 2 | 2 | 9 | 37 |
| " 251 | 1 | 2 | 2 | 14 | 3 | 2 | 2 | 9 | 37 |
| PK 1-2-25-2: Cell 278 | 2 | 1 | 2 | 14 | 4 | 1 | 4 | 8 | 37 |
| " 278 | 2 | 1 | 2 | 15 | 4 | 1 | 4 | 7 | 37 |
| " 269 | 2 | 1 | 2 | 14 | 4 | 1 | 4 | 8 | 37 |
| " 275 | 2 | 1 | 2 | 15 | 4 | 1 | 4 | 7 | 37 |
| " 262 | 2 | 1 | 2 | 15 | 4 | 1 | 4 | 8 | 37 |
| " 267 | 2 | 1 | 2 | 14 | 4 | 1 | 4 | 8 | 37 |
| PK 1-2-26-3: Cell 301 | 1 | 2 | 3 | 15 | 3 | 3 | 3 | 6 | 38 |
| " 300 | 2 | 2 | 3 | 13 | 4 | 2 | 3 | 7 | 38 |
| " 298 | 2 | 2 | 3 | 13 | 4 | 2 | 3 | 7 | 38 |
| " 295 | 2 | 2 | 3 | 13 | 4 | 2 | 3 | 7 | 38 |
| " 293 | 2 | 2 | 3 | 12 | 4 | 2 | 3 | 7 | 37 |
| PK 1-2-45-2: Cell 287 | 2 | 2 | 3 | 15 | 4 | 1 | 5 | 6 | 39 |
| " 289 | 2 | 2 | 3 | 14 | 4 | 1 | 5 | 6 | 38 |
| " 291 | 2 | 2 | 3 | 15 | 4 | 1 | 5 | 6 | 36 |
| " 282 | 2 | 2 | 3 | 14 | 4 | 1 | 5 | 6 | 38 |
| PK 1-2-51-1: Cell 365 | 2 | 1 | 3 | 15 | 3 | 2 | 2 | 7 | 37 |
| " 366 | 2 | 1 | 3 | 15 | 3 | 2 | 2 | 7 | 37 |
| PK 1-2-65-1: Cell 326 | 2 | 2 | 5 | 18 | 3 | 2 | 1 | 4 | 38 |
| " 319 | 2 | 2 | 5 | 13 | 3 | 2 | 2 | 7 | 37 |
| " 323 | 2 | 2 | 5 | 13 | 3 | 2 | 2 | 7 | 36 |
| " 322 | 2 | 2 | 5 | 13 | 3 | 2 | 2 | 7 | 37 |
| " 318 | 2 | 2 | 5 | 14 | 2 | 2 | 2 | 7 | 37 |
| " 315 | 2 | 2 | 5 | 13 | 3 | 2 | 2 | 7 | 37 |

Note: Large, constricted chromosomes shown in idiograms are grouped in G-IV.
between sister clones have been demonstrated above, as in the case of PK 1-8 and in the marker lines. Similar results have been reported by Chu and Giles (3). Mosaic clones may also appear whose several components have arisen from the parental population through faulty cloning (6).

When variant clones appeared from populations in which the variant types were not detectable, another possible explanation of clone variation arises. This is that new variant types developed in young clone populations, and, as such populations grew larger, these variants became established as population components. Variation referable to such a process has been described above in the case of PK 1-2-23 and PK 1-2-65. The small numbers of cells in early clones might be conducive to this, since mechanisms which tend to deter the increase of variant types in large populations such as sequential selection as described by Braun (2) for bacteria would not be operable. In addition, small populations may be under quite different selective pressures, because of less heterostasis in terms of equilibrium between cells and medium. Thus, variation in and between sister clone populations may be attributed to the cloning procedure itself as well as the selection of pre-existing variants in the parental population. This makes it necessary that clone populations be checked for uniformity as they arise. It should not be assumed that a population is homogeneous because it has been derived from a single cell.

The question of population homogeneity has not been given sufficient attention by experimentalists who employ cell populations. Cell cultures are widely used as assay systems in such fields as virology, nutrition, and cancerology. Many studies have been performed with insufficient information on the composition of the test systems, making for the misinterpretation of experiments. For example, some results could be attributed to complex populations of multiple subcomponents rather than experimental design. Further, reproducibility of results has been impaired in some instances because of rapid population changes or differences between supposedly like cell populations from different laboratories. Such possible errors can be minimized or avoided by a characterization of cell strains in terms of their chromosomes or other indicators. It is assumed here that mutation-selection occurs generally in tissue culture systems, and the following remarks will be made especially within this conceptual framework.

Studies on the chromosomes of stable lines show that populations may be either entirely heteroploid (10, 17) or in exceptional instances very nearly unaltered diploid (15, 19). Other studies which have been made on newly explanted cells have shown that conversions of diploid to heteroploid usually occur early in cultivation and with relative rapidity (11). The PK lines showed an intermediate condition in the usual transformation to heteroploidy. The main component was quasi-diploid, with only a few departures from the normal diploid karyotype. A small quasitetraploid component was present, together with a larger subtetraploid or heteroploid component. Cloning has shown that the heteroploid component could be cultivated separately, thus indicating its autonomy. It is of interest that quasi-diploid and heteroploid components could continue in mixed condition for a long period of time. This suggests that adaptations, probably in the form of mutation-selection, can occur in the quasi-diploid components as well as in the components of higher ploidy which have been considered as genetically more versatile by Levan (9).

Puck (15) has stated that medium is of prime importance in stabilizing diploid components. It is logical that if the tissue culture environment were suited to the growth of diploid cells there would be a greater probability of adaptive mutants arising among these cells, thus perpetuating the diploid condition. However, it should be pointed out that PK lines have been cultivated on a variety of media. Thus factors such as the inherent genetic adaptability of particular cell types and the chance acquisition of adaptive mutations must be considered as well as the enrichment properties of the nutrient medium.

It should be kept in mind that tissue culture media cannot be expected to reproduce exactly the conditions of the in vivo environment. It follows that certain variants will be selected and that stable populations even of cytologically diploid cells should be suspected of being genetically diverse and different from the cells of origin.

ACKNOWLEDGMENT

The author wishes to express hearty thanks to Professor Morgan Harris for his helpful advice and criticism during the course of this work.

REFERENCES


6. HARRIS, M., and RUDDLE, F. H. Growth and Chromosome Studies on Drug Resistant Lines of Cells in Tissue Culture. (14th Annual Symposium on Cancer Research under the auspices of the University of Texas M. D. Anderson Hospital and Tumor Institute, 1960.)


Fig. 1.—Photo-idiogram of the normal male Hamshire pig chromosome complement from primary kidney culture. The symbols in parentheses refer to easily distinguished morphological groups (G). The arrow points to one of the G-VI chromosomes which has been lengthened because of overlapping by another chromosome. The box contains chromosomes from another cell whose G-V and Y chromosomes are particularly clear.
FIG. 2.--Photo-idiograms of PK 1-0-0. In cell A the various morphological groups which relate to those in the normal Hampshire karyotype are indicated. In cell B a chromosome (*) in G-VIII is obscure because of its lying near debris. A centric fusion (CF) which has occurred between a G-VI and a G-VII chromosome is shown in cell B.

FIG. 8.--Photo-idiograms of PK 1-2-0-0. The marker properties of this line are conferred by one G-I chromosome with a shortened long arm and a chromosome which is intermediate in length between the G-VI and G-VII chromosomes. Two extra G-VIII chromosomes are present. Cells A and B are identical.

FIG. 4.--Photo-idiograms of PK 1-0-3-0. The marker chromosome (M) is the product of a translocation between a G-VI and a G-III chromosome. Note that, while the chromosome numbers of cells A and B are identical over-all, differences occur between G-IV and G-VIII.
FIG. 5.—Photo-idiograms of PK 1-2-36-8. Two marker chromosomes are present. One ($M_1$) is a subacrocentric type of chromosome with a secondary constriction. The other ($M_2$) is a minute chromosome of the acrocentric type. The markers have probably arisen through a translocation between a G-VII (through the centromere) and a G-IV chromosome. Cell A has six G-VIII chromosomes, whereas B has seven. Cell A has thirteen G-IV chromosomes, but one of these could possibly belong to G-V. Cell B, on the other hand, has only eleven G-IV chromosomes.

FIG. 6.—Photo-idiogram of PK 1-2-43-3. The marker ($M$) is an exceedingly long acrocentric chromosome. The chromosome numbers of cells A and B are different, there being one G-II lacking in cell B.

FIG. 7.—Photo-idiograms of PK 1-2-65-1. The marker ($M$) is the result of a centric fusion between two G-VII chromosomes. The difference between cells A and B may be due to the difficulty of placing one chromosome in either G-IV or G-V.
FIG. 5.—Photo-idiograms of PK 1-2-26-3. Two marker chromosomes are present. One ($M_1$) is a subacrocentric type of chromosome with a secondary constriction. The other ($M_2$) is a minute chromosome of the acrocentric type. The markers have probably arisen through a translocation between a G-VII (through the centromere) and a G-IV chromosome. Cell A has six G-VIII chromosomes, whereas B has seven. Cell A has thirteen G-IV chromosomes, but one of these could possibly belong to G-V. Cell B, on the other hand, has only eleven G-IV chromosomes.

FIG. 6.—Photo-idiogram of PK 1-2-43-3. The marker ($M$) is an exceedingly long acrocentric chromosome. The chromosome numbers of cells A and B are different, there being one G-II lacking in cell B.

FIG. 7.—Photo-idiograms of PK 1-2-65-1. The marker ($M$) is the result of a centric fusion between two G-VII chromosomes. The difference between cells A and B may be due to the difficulty of placing one chromosome in either G-IV or G-V.
Chromosome Variation in Cell Populations Derived from Pig Kidney

Frank H. Ruddle


**Updated version**
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/21/7/885

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.