Morphological Transformation in an Established Line of Pig Kidney Cells.* (27120)

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It is known from chromosome studies that variant cell types may arise in highly homogenous populations, and further that such variants can give rise to large populations of cells(1,2,3,4). Genetic variation in terms of chromosomal rearrangements or point mutations must be considered as relatively frequently occurring events in cell populations. It is conceivable that such variations will evoke cell types uniquely characteristic in terms of their morphology. A number of investigators have reported the spontaneous appearance of morphological variants whose characteristics perpetuated themselves in stable fashion(5,6). However, these particular transformations have been shown due to contamination with cells of a foreign origin (7,8). Such developments have cast perhaps unnecessary doubt on other reports of cell alterations(9,10,11), but have properly shown the need for criteria in assessing morphological transformation *in vitro*. A spontaneous and stable conversion in cell morphology was observed to occur in this laboratory in clones of cells whose chromosomal properties were then being studied. Fortunately each of these lines carried unique chromosomal markers which allowed exclusion of the usual argument of cellular contamination.

Observations. The clones in which the alterations occurred arose from an established line of pig kidney cells whose history, details of cultivation, and general characteristics are described elsewhere(4). The 2 clones have been designated PK 1-2-51-1 and PK 1-2-43-2. PK 1-2-51-1 which had grown as a tightly contiguous epithelioid cell sheet showed the presence of stellate, individualized cells after 8 months of continuous cultivation. The cells appeared at first in small numbers, nestled in the gaps and spaces in the cell sheet, and within 2 months' time the cultures were predominately composed of this cell type. A similar transformation occurred in PK 1-2-43-2 which began to evince the presence of rounded cells following 11 months of continuous cultivation (Fig. 1 and 2). The rounded elements increased until they became the dominant cell type, but patches of epithelioid cells continued to remain. Chromosome studies of both cultures before and following morphological alterations showed all metaphase cells to possess uniformly a marker chromosome characteristic of each of the respective lines (Fig. 3 and 4). This indicated that transformation could not be explained on the basis of contamination by foreign cells.

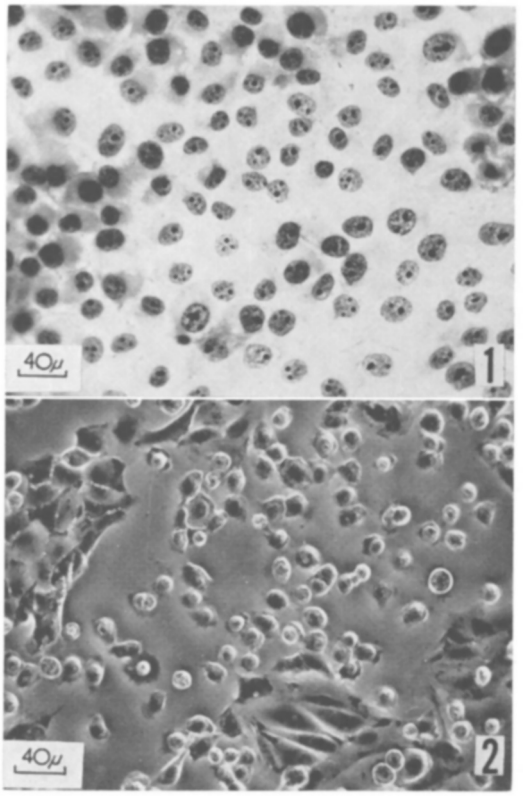

FIG. 1. Contiguous epithelioid cells of PK 1-2-43-2. H and E.

FIG. 2. Non-contiguous round cells and contiguous epithelioid cells of PK 1-2-43-2. Phase contrast.

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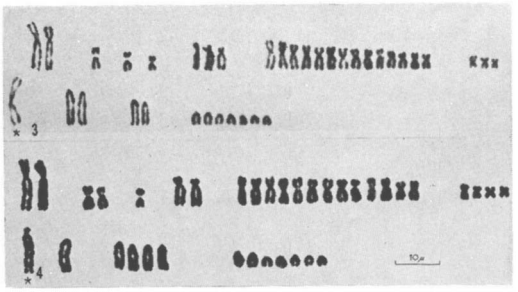

FIG. 3. Photo-idiograms of PK 1-2-51-1 showing the long metacentric marker chromosome(*).
FIG. 4. Photo-idiogram of PK 1-2-43-2 showing the long subacrocentric marker chromosome(*).

In PK 1-2-43-2, the population was studied in finer detail by examining clones which were obtained after transformation (Chart 1). Both round and epithelioid cell types were cloned and cultured in pure form as mass cultures. The morphological characteristics remained stable over a period of 2 months when it was necessary to terminate this work. Round cell clones greatly outnumbered epithelioid clones, but the former tended to grow poorly at low cell concentrations and therefore were difficult to pick and grow to large populations. Five epithelioid clones had chromosome counts whose modal values were 43 in 4 instances and 48 in a fifth. One clone whose modal value was 38 was difficult to class morphologically. Its cells were small and tended to round up, but also grew contiguously and formed sheets. Other properties such as high cell yield per flask and rapid alteration of medium pH tended to align this strain with the round cell types rather than the epithelioid. Therefore, it appears on the basis of these few clones that round cells may have differed from epithelioid cells not only on the basis of morphology, but also on the basis of chromosome number. There is however no evidence that alteration of chromosome pattern was causal to alteration of cell morphology. In fact, since the 43 chromosome component was observed before the appearance of the round cell variant, and since the round cell variant arose in the parental 38 chromosome component, it is most probable that this morphological variant was not causally related to its chromosome number.

Discussion. When cells are freshly cultivated as primary cultures, it is known that after a variable period of time they degenerate and die, or following a period sometimes marked by some obvious form of transformation continue to proliferate in a more or less continuous fashion. This process of early alteration has been characterized chiefly in terms of morphology, rate and mode of growth, and karyotypic alteration. Because of its usually abrupt and stable nature, and because variants first appear as small foci, primary transformation has been considered as causally related to mutational events. Interestingly, early transformations have properties which relate them to alterations which occur later in established cell strains, such as those reported here. There may be only slight difference between the two in terms of mechanism. However, they may serve slightly different functions in that while primary transformations involved adjustment to *in vitro* conditions, secondary transformations may confer advantageous adaptive characteristics such as heightened growth rate, ability to maintain high growth rate at low pH, continued growth under crowded conditions, and so on. While mutation-selection arguments seem valid in explanation of cellular alterations, other mechanisms are possible. One which has fairly broad observational basis is infection by latent viruses. A number of workers have demonstrated that infection of cells with Rous(12) and polyoma(13) viruses can produce cell morphology changes which are very similar to those described here but do not necessarily give rise to long term lines. It is also of interest that these alterations, while associated with a malignant proc-

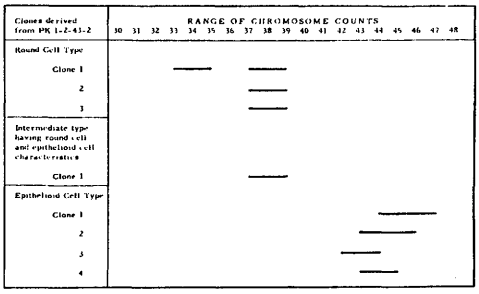

CHART 1. Comparison between cells of different morphological type and chromosome number.

ess, do not involve chromosomal rearrangements. It is possible that similar viral agents accidentally introduced *via* serum or tissue extracts could produce similar results in established cell lines.

In conclusion, it should be realized that morphological changes can occur in cell populations, and that they are not necessarily due to contamination by foreign cells. Such changes offer opportunities for study of the population dynamics of *in vitro* cell populations.

Summary. Morphological transformations occurred in 2 clone populations of pig kidney cells. Both populations carried distinct chromosomal markers whose incidence and type did not alter during transformation. Thus, the alteration of morphology was intrinsic and could not be due to cell contamination. In one clone, a change in chromosome number seemed to be related to the appearance of a new morphological type.

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Relationship of Serotonin and Reserpine in the Chicken. (27121)

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Numerous effects have been ascribed to the use of reserpine as a poultry feed additive. Reports on average daily gain and feed efficiency have been inconsistent(1-5). Experiments have been conducted to determine the sedative action of reserpine in the hope of obtaining a drug that will reduce economic losses caused by undue excitement of the poultry flock. The results of such tests were inconclusive(2,4,5). Effects of orally administered reserpine on egg production and quality have been reported(6,7). Low levels of reserpine in the diet increased egg weight and shell quality during periods of high environmental temperatures. No effect on albumen quality was seen.

Inconstant results obtained in feeding tests may be related to many factors, *e.g.*, dose, duration of intake, age of animal, and diet. Carlson(8) suggested that poultry rations low in tryptophan, a precursor of serotonin (5-hydroxytryptamine), might result in growth depression as a result of adding reserpine to the feed. Although many reserpine-feed additive tests have been conducted, the pharmacodynamics of reserpine in poultry has not been extensively studied.

Sturkie *et al.*(9) investigated the physiologic effects of reserpine on the chicken and observed that it produced a significant hypothermia, bradycardia and a decrease in mean arterial pressure when administered intramuscularly at levels of 0.1 to 0.2 mg/kg. This level of reserpine produced diarrhea.

Premachandra *et al.*(10) investigated the relationship of parenterally administered reserpine and thyroid activity in chickens. No effect on thyroid uptake of I^{131} was observed.