any effect in weight gain could be produced by feeding a seriously overoxidized product.

Weanling rats (Wistar-Purdue strain) were fed daily 5 g of a highly nutritious basal diet (*5*) supplemented by 1 or 2 g per day of the starch products under investigation. The basal diet (5 g/day) was sufficient to produce a weight gain of 4 to 8 g per week during 4 weeks.

The experiment was conducted in two parts, in each of which nine diets were fed. These were as follows: one basal diet of 5 g/day; four supplemented diets containing 5 g of basal diet and 1 g of a starch product, or a total of 6 g/day; and four supplemented diets containing the basal diet and 2 g of a starch product, or a total of 7 g/day.

The nine diets were arranged in a randomized incomplete block design (*6*), and each diet was replicated four times. Equal numbers of male and female weanling rats were grouped (three litter mates per block, three blocks per replication), individually caged, and fed 5.0 ± 0.1 g per day for 7 days. Water was freely supplied. At the end of this period the animals were weighed and either continued on the basal diet or changed to diets composed of basal diet mixed with 1 or 2 g of starch product supplement. Animals were weighed again after 3, 7, 14, and 21 days of supplementation.

Responses to supplementation were so uniform that 7-day weights reflected the increased caloric intake as definitely as weights at 14 days or 21 days. The weight gains at 21 days for the rats in the two groups of the experiment are presented in Table 1. The data for each group were analyzed in a 4 by 2 factorial design: four types of starch fed at two levels. This anaylsis permitted evaluation of the effect of the amount of supplement, the type of supplement, and the interaction of type and amount.

All the commercial starches produced weight gains similar to corn starch (Table 1). However, starch *c*, heavily oxidized and degraded by 2 equivalents of hypochlorite per D-glucose unit (43.2 percent, wt./wt., of chlorine) had a low nutritional value since the average weight gain produced by feeding this starch was significantly lower than all the others. The average weight gains produced by supplements of 1 and 2 g of this starch were not very much different from that produced by the basal diet (a gain of 26.7 g during 21 days).

The average weight gain produced by all 2-g supplemented diets was significantly greater than that produced by 1-g supplemented diets, the difference being about 10 g per rat in 21 days. There was no interaction between the amount of supplement and type of starch; this is shown by the fact that doubling the amount of supplement increased the gain about equally for all the starch products. There was close agreement between the weight gains reported for corn starch in groups 1 and 2. This similarity allowed a comparison of weight gain between products in the two groups. Heavily oxidized noncommercial starch (*c*) induced diarrhea after the 2nd day of supplementation. Hydroxyethyl starch produced a mild diarrhea.

Autopsies performed on one rat from each of the 2-g supplemented diets disclosed that rats fed heavily oxidized starch *c* had a marked dilation of the colon. The other animals appeared to be normal (*7*).

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Maintenance of Normal in situ Chromosomal Features in Long-Term Tissue Cultures

Abstract. Clonal isolates from a rapidly proliferating fibroblast-like derivative have retained the classic diploid chromosome relationship over a period of many transplant generations. The readily identified members of the 11 pairs of chromosomes, including the sex chromosomes (X_1 and X_2), have aided in localizing minute structural alterations within recloned diploid and aneuploid sublines.

An extensive search to isolate stable diploid tissue cultures of the Chinese hamster (*1*), which continually display the chromosomal state noted *in situ* (*2*), has been wholly successful. The twenty-eighth growth in the series, stemming from adult female fibroblast-like derivatives (FAF-28), possessed the many desired features that characterize the classic diploid state (Fig. 1). The strain was derived from a population of normal cellular components that had infiltrated the peritoneal cavity of an animal bearing tumor CH-38MC, a 3-methylcholanthrene-induced fibrosarcoma. This scheme to initiate cultures was routinely employed, since tetraploid tumor cells always failed to attach to the surface of the flask, leaving normal cellular infiltrates to proliferate for varying lengths of time and, in some cases, without undergoing the eventual shift toward aneuploidy. Generation times gradually shortened as sublines progressed into the tenth month, at which time single-cell cloning trials were conducted in an effort to isolate the increasing number of classic tetraploids. Current parental and clonal derivatives proliferate very rapidly (14 hours or less) in a variety of chemically defined media prepared with whole serum (*3–5*).

Plating efficiencies of three classic diploid and one subdiploid variant, isolated by a modification of procedures described by Puck *et al.* (*5*), ranged from 20 to 40 percent, 7 to 12 days after 60-mm petri dishes were seeded with 500 to 2000 single cells, and in the absence of subsequent and recommended changes of the medium. Precautions regarding serum toxicity and prescribed handling of glassware were neglected during these preliminary trials. FAF-28 exhibits very few spontaneous chromosome breaks. Aneuploidy, that is, a ±1 chromosome deviation from the euploid number, rarely exceeds 20 percent of the cells seen in division, as in the case of intact bone marrow, regenerating liver, and corneal epithelium. Tetraploidy in FAF-28 has fluctuated from 1 to 25 percent of the mitotic population and has consisted primarily of classic tetraploids arising from endoreduplication. It appears that repeated trypsinization of rapidly proliferating classic diploid cells causes dividing metaphases to undergo restitution when suddenly detached and transferred to the new culture flask. Somatically paired homologues are seen more frequently early in the next transfer generation.

Although the later parental FAF-28 sublines were characterized by rigid classic diploidy and tetraploidy, single-cell clonings yielded an unexpected array of aneuploids and even near-triploids. The second cloning trial failed to reveal triploids. On both occasions, classic tetraploid cell types failed to clone. Parental-like classic diploid sublines were repeatedly isolated

by cloning procedures and represented 75 percent (20/26) or better of the clones selected at random. Five other clones (20 percent) featured minor anomalies, such as quasidiploid, hypodiploidy, and hyperdiploidy, and one clone (5 percent) was a near-tetraploid. Thus, the intact or normally observed in vivo pattern of chromosomes was reflected uniformly among in vitro clonal derivatives.

Single-cell cloning of an otherwise classical diploid-tetraploid parental population was demonstrated to be a fertile source from which to isolate cytologic mutants. The ease with which variants proliferated during the clonal period may be a direct response to the greatly reduced numbers of cells employed during cloning and the accompanying release from pressures exerted collectively by large numbers of stem cells. Elimination of the latter may have been instrumental in providing genetically altered forms an opportunity to compete favorably with the sparsely settled classic diploids.

Puck, on several occasions, has commented that plating efficiencies of normal primary explants are exceedingly low and, therefore, inappropriate for use in controlled replication of quantitative studies. The absence of data on plating efficiencies of other long-term euploid cultures of human beings and opossums limits this brief discussion (*6*). Karyological "break-down," resulting in the formation of predominantly aneuploid stemlines (with or without new chromosomes), has been witnessed repeatedly during the course of establishing other strains (*4*, *7*). Similar trends have yet to be noted among the many clonal sublines of FAF-28; the normal *in situ* chromosome relationships are constantly expressed. This is presumably because of the exceedingly rapid proliferative rate of the current and exceptional classic diploid stem cells. When aneuploid or quasidiploid forms predominated in other rapidly proliferating strains, the parental euploid cells were comparatively slow in dividing, thereby encouraging the shift to aneuploidy. One may ask if the rapid proliferative ability of FAF-28 could be accompanied by other undisclosed stem-cell features that reflect greater selectivity when competing with potential mutants. Consequently, single-cell cloning of FAF-28 provides the only opportunity to isolate spontaneous mutation which, otherwise, would be eliminated during routine propagation in the presence of larger numbers of the exceptional euploid stem cells.

Because of these features, FAF-28 is an unusual and rare cell type and one that is highly desirable for experimentation. Its unique features are most applicable in a variety of studies pertaining to somatic cell genetics in vitro.

Parental and clonal sublines of FAF-28 are currently being employed in a series of trials to characterize the responses to x-ray and ultraviolet radiation, production of mutants (genic and chromosomal) by means of selective inhibitory actions of antimetabolites and viral susceptibilities, and to attempt a disclosure of distinctive cytological features that may accompany "malignant transformation," after in vivo implantations (*8*).

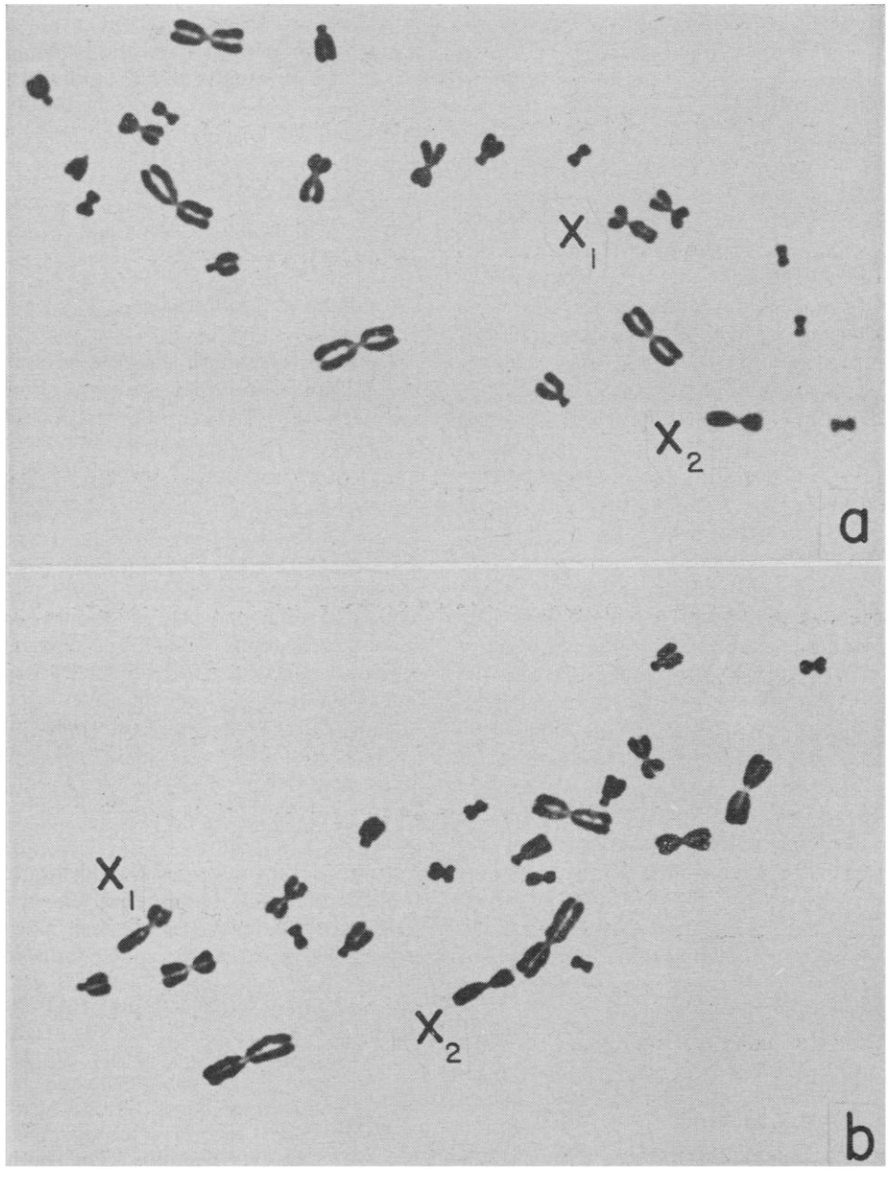

Fig. 1. Classic diploid complement of chromosomes ($2n = 22$) of FAF-28. Note the replicable dual appearance (X_1 and X_2) of the X-chromosome pair, as proposed by the *Triheterosomic* scheme for mammalian sex determination (X_1X_2Y).

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