and epinephrine throughout the body supplies a potent tool for studying the role and interplay of these neurohormones in the brain as well as peripherally.

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Isolation of a Nutritional Variant from a Culture of Rabbit Fibroblasts

The results of earlier studies dealing with the cultural characteristics of rabbit fibroblasts derived from normal tissue indicated that the over-all nutritional requirements of newly established strains vary as a function of their age *in vitro* (*1*). The quantitative aspects of the data were consistent with the concept that either a limited number of cells in the original population (inoculum) were capable of continuous growth in the medium employed or that such cells appeared in the population as a result of mutation. The present experiments (*2*) were undertaken to obtain information regarding the fundamental question of whether nutritional variants are present in a given culture after prolonged serial cultivation *in vitro*. Rabbit fibroblasts, strain RM3-56, which had been propagated serially *in vitro* for almost 2 years were selected for study (*1, 3*). On the assumption that the proportion of nutritional variants in a particular population might be too small to be detected by examining the progeny of single cells selected at random from the population, it was considered expedient to establish conditions which favor the proliferation of variants by altering the composition of the medium.

Table 1. Isolation of a nutritional variant from a culture of RM3-56 fibroblasts.

No. of passages *in vitro*	No. of cells*	No. of colonies†
79	1.1×10^6	1
87	0.9×10^6	0
91	1.5×10^6	2

* Second passage in medium 73.
† Colonies containing cells capable of continuous proliferation in medium 73.

The following media were employed: medium 56 was 5 percent chick embryo extract, 10 percent normal horse serum, and 85 percent S18 (volume for volume); medium 73 was 2 percent dialyzed horse serum, 98 percent S16. Solutions S16 and S18 are of similar composition and contain amino acids, vitamins, salts, and glucose (*3*). The procedure employed for propagating RM3-56 serially in medium 56 and the methods used in quantitative experiments were described previously (*3*). The experiments were based on the observation that RM3-56 fibroblasts fail to proliferate in medium 73 after the first subculture. Under these conditions, the nonproliferating cells gradually degenerate over a period of from 3 to 4 weeks. The regular schedule of fluid replacements was continued, to permit the detection of any cells capable of proliferating in this medium. The results of three experiments performed with cells of different ages *in vitro* are summarized in Table 1.

In the first and third experiments, three colonies, containing from 50 to 100 healthy fibroblasts, were observed after 9 to 16 days (second passage in medium 73). These cells proliferated rapidly, whereas the remaining fibroblasts degenerated. In the second experiment, none of the cells appeared capable of proliferating in medium 73. The cells comprising one of the colonies were subsequently subcultured, and their progeny continue to proliferate after 40 passages in medium 73. This variant or subline is designated RM3-73, to indicate both the tissue from which the culture was originally derived and the medium in which the strain is propagated. Strain RM3-73 also differs from RM3-56 in that it proliferates at a somewhat slower rate in medium 56. On the other hand, strain RM3-73 is indistinguishable from RM3-56 by such criteria as morphology and susceptibility to infection with vaccinia virus.

Of particular significance is the fact that only a small fraction of the cells in RM3-56 cultures are of the 73-type. For example, the three colonies isolated in experiments (Table 1) that employed 3.5×10^6 cells contained a total of only 225 cells when first observed. The proportion of RM3-73 cells in a population of RM3-56, calculated on this basis, is a maximal value because the 73 cells were proliferating throughout the course of the experiments. It is apparent that, under conditions where the ratio of two nutritional types of cells is less than 1 to 10^4, cloning techniques (*4–6*) are impractical for detecting the minority type. The method of selection applied under conditions where a large proportion of the cells fail to proliferate provides a practical means for studying large populations of cells with respect to nutritional cell types. Furthermore, the presence of nonproliferating cells serves the same purpose, in permitting isolated cells to proliferate, as the addition of irradiated cells ("feeder layer") described by Puck and Marcus (*5*). The method is particularly suitable for studying the genetics of cell lines whose nutritional requirements have been partially defined. In these instances, by combining the selection method and cloning techniques, it becomes possible to study somatic cells with any of the required nutritional factors as genetic markers. It is anticipated that the availability of a broad spectrum of nutritional cell types not only will be of aid in understanding the physiology of cultured cells but will enhance the usefulness of the tissue-culture method in virology and in studies on malignancy.

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Histochemical Demonstration of Enzymes Separated by Zone Electrophoresis in Starch Gels

Zone electrophoresis in starch gels is a rapid, simple, dependable method of high-resolving power for separating complex mixtures of proteins. We have combined this method with histochemical techniques for locating and identifying enzymes in tissue sections in order to analyze the enzymatic composition of biological material (*1, 2*). The numerous esterases responsible for the high level of esterase activity in the mouse liver