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* Present address: Department of Physiology and Biochemistry, Medical College of Pennsylvania, Philadelphia 19129.

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## HeLa Marker Chromosomes, Chang Liver Cells, and Liver-Specific Functions

Nelson-Rees and Flandermeyer have (*1*) indicted the Chang liver cell (*2*) as a HeLa cell contaminant. They have concluded that, regardless of designation, the Chang cell should be considered a de facto strain of HeLa. These authors based their conclusion on the following: (i) the electromobility of glucose-6-phosphate dehydrogenase (G6PD) and phosphoglucomutase of the Chang cell were similar to those of the HeLa cell; (ii) the Chang cell contained a complex of rearranged chromosomes or markers described for HeLa cell cultures; and (iii) there was no Y chromosome in the Chang cell. It is unfortunate that these authors failed to mention that Kaighn and Prince (*3*) had found the Chang but not the HeLa cell capable of producing serum albumin and fibrinogen, and that Bausher and Schaeffer (*4*) had demonstrated tyrosine aminotransferase activity in the Chang cell.

In 1953 to 1954 I made many attempts to cultivate cells from a variety of human tissues on the simplistic assumption that a specific differentiated cell might support in vitro the growth of a specific virus (for example, the human hepatocyte might support the growth of the human hepatitis virus). Since the goal was merely to obtain a sufficient number of cells that would support the growth of certain viruses under study, no effort was made to record the sex, race, age, and medical diagnosis of the tissue donor. The liver specimen, from which the Chang cell was derived (*2*), was obtained during biopsy from a patient undergoing exploratory laparotomy.

Since there is no record of the sex and race of this tissue donor, the absence of a Y chromosome and the presence of G6PD and phosphoglucomutase with specific electromigration patterns (similar to those found predominantly among the black race) cannot be used as evidence for indicting the Chang liver cell, because the tissue donor could be a black woman. Therefore, the indictment by Nelson-Rees and Flandermeyer is based solely on the morphologic appearance of chromosomes.

Ludueña *et al.* (*5*) have presented evidence that another protein characteristic of, but perhaps not unique to, differentiated liver cells (liver alkaline phosphatase) is synthesized by the Chang but not by the HeLa cell. There are now on record three groups of investigators who have found proteins characteristic of differentiated human liver cells in or secreted by the Chang liver cell. Other reported differences between these two cell lines include susceptibility to aflatoxin $B_1$ (*6*) and the total and epinephrine-sensitive adenyl cyclase activities (*7*).

In view of these reports, I ask the following questions: If the Chang cell is derived from a culture of HeLa cell and not from a human liver biopsy as reported (*2*), what is the probability that the Chang but not the HeLa cell contains more than one liver-specific protein? Is chromosomal morphology sufficiently dependable to be used as the sole criterion in tracing the origin of an established line of human cells? We are all aware of the seriousness of cross-culture cell contamination in research involving cell cultures. But, to indict a cell line as a HeLa cell contaminant on insufficient evidence may be counterproductive.

R. SHIHMAN CHANG
*Department of Medical Microbiology, University of California, Davis 95616*

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"Chang liver cells" were first suspected of being HeLa cells in 1966 on enzymatic grounds (*1*). In 1974, Chang brought to my laboratory a culture of the "liver cells" for karyologic analysis. Our results were discussed with Chang and with Sussman, a co-author of the paper by Ludueña cited by Chang in his comment, and were summarized by us (*2*). Our results were confirmed by Lavappa *et al.* (*3*) on the "Chang liver cells" at the American Type Culture Collection (ATCC) (*3*). We indicted the cells as being HeLa cell contaminants because they possess a group of chromosomes originally described by Miller *et al.* (*4*) for HeLa cells. These "markers" consist of chromosomes whose banding patterns coincide with those of portions of specific human chromosomes, however rearranged (translocations, misdivision, nondisjunction). Besides these "Miller markers," many HeLa strains share other identical markers which serve to characterize closely related strains of HeLa or the culprits in HeLa contamination of other cultures. In fact, we communicated to Chang that some markers that we observed in the "liver cells" were identical to some observed in the HeLa-contaminated cultures from Russian laboratories (*5*). In every culture analyzed to date, the cells that exhibit "Miller markers" and others also lack a Y chromosome and produce type A (fast moving) G6PD.

A sample of "Chang liver cells" supplied by the ATCC was studied recently by O'Brien (*6*) for additional enzyme polymorphism. The cells exhibited characteristics identical to HeLa and to three other now well-known HeLa strains—H.Ep-2, KB, and J111—in the electrophoretic resolution of seven relatively polymorphic, human gene-enzyme systems previously studied by him [see (*7*)]. According to O'Brien the genotype frequency of HeLa, based on allelic frequencies of the seven tested enzyme loci in natural populations, is 0.013; or, as concerns all cells studied by him, the probability that another cell line would express the same genotype is .05.

Thus, while there is no record of sex, age, race, and medical diagnosis of the tissue donor for the original liver culture, the results of up-to-date karyology and enzymology speak more convincingly for its being now a strain of HeLa cells through the common occurrence of cross-cell-contamination than that of a liver derivative of which no other human line exists in spite of many initiation attempts. As to the liver functions detected in this strain of HeLa, we have re-


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cently publicized a variety of products elaborated to a greater or lesser extent and unexpectedly by different strains of HeLa. Briefly, two purported "breast carcinoma" lines, G-11 and HT-39, produce "$\alpha$"-lactalbumin (*8*); a "prostate adenoma," MA160, shows $C_{19}$-radiosteroid metabolism of prostatic epithelium (*9*); "lung tumor cells," 2563 (= MAC-21), produce antibodies in rabbits that act specifically against human lung tissue (*10*).

A hypothesis of genetic derepression seems to be the simplest explanation for these results in variants of HeLa cells, derived as they probably were from a single cell. While certain physical characteristics (marker chromosomes) remain virtually unaltered, and many genetically determined gene-enzyme systems remain constant in all HeLa cells, some strains of these aberrant human cells are generating unexpected products similar in a sense to "ectopic" behavior of tumor cells in vivo. Alternatively, one may be detecting fetal antigen activity.

It appears that a single culture of HeLa cells cannot now be considered as the sole representative of this vast family of cell strains in terms of products; a condition which may well be the fate of all bona fide long-term cultivated cell lines regardless of tissue of origin.

WALTER A. NELSON-REES
*University of California,*
*Naval Biosciences Laboratory,*
*Oakland 94625*

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## Membrane Potentials of Mitochondria

Maloff *et al.* (*1*) claim that "the membrane potentials of giant mitochondria from cuprizone-fed mice were found to be independent of metabolic state." This conclusion, which is based on the use of microelectrodes, is in sharp contrast to a large body of evidence obtained from various mitochondrial systems by a variety of techniques [see (*2*)]. Maloff *et al.* also claim that "experiments are described in which the presence of the microelectrodes in the inner mitochondrial space, . . . are validated." The only experiment brought as evidence for the latter claim is the response of the microelectrode signal to the addition of valinomycin and NaSCN. The potential which is positive initially is not affected by the addition of succinate but is slowly reversed by the addition of valinomycin and further reversed by the addition of NaSCN [see (*1*, figure 1A)]. Maloff *et al.* argue that the reversal of the polarity is the result of the formation of a diffusion potential across the mitochondrial inner membrane, the inner space of this membrane having internal $K^+$ and no $SCN^-$ anion. Thus, they argue, valinomycin (which increases membrane permeability to $K^+$) induced a diffusion potential across the inner membrane, the inner space thus being negative. Hence, the reversal of the electrode polarity indicates its location in the mitochondrial matrix. Similarly, the addition of the lipophilic anion $SCN^-$ would induce diffusion potential across the inner membrane, making the inner space negative. I would like to point out that the microelectrode tip also has a very high potassium concentration (2*M*) and no $SCN^-$ anion. Thus, for instance, if the electrode tip became obstructed by a membrane or hydrophobic material, valinomycin (and $SCN^-$) would induce the formation of a negative potential. A membrane-coated microelectrode is very much like a $K^+$ specific membrane-electrode or other ion-specific membrane electrodes. In fact it is not unlikely that the inner membrane of the mitochondria, which has a very large surface to volume ratio and is not a rigid sphere, simply engulfs the electrode tip without being penetrated and thus converts the electrode into an ion-specific membrane electrode. In this event the electrode responds to valinomycin and $SCN^-$ but not to any metabolic event in the mitochondrion. Recent experiments with both intrinsic and extrinsic membrane potential probes (*3*) have indicated that unlike the experiments of Maloff *et al.* (*1*), $K^+$ diffusion potentials when induced by valinomycin in nonenergized mitochondria are formed within 1 second and decay fairly rapidly with a half-time of 10 to 30 seconds. This decay is due first to the movement of other ions, mostly protons, in exchange for $K^+$ and second to the consequent slower depletion of the mitochondrial $K^+$. Moreoever, after an addition of succinate the potential which is generated by the proton pump is negative and high (*4*), and the addition of valinomycin, in the presence of external $K^+$ (5 m*M*), decreases the high negative value by about 50 mv opposite to what Maloff *et al.* (*1*) observed. This effect is due to the influx of $K^+$ into the matrix in contrast to the euflux which is generated in nonenergized mitochondria. Thus, it is suggested that Maloff *et al.* do not measure potentials across the inner mitochondrial membrane but probably across the obstructed electrode tip.

HAGAI ROTTENBERG
*Bell Laboratories,*
*Murray Hill, New Jersey 07974*

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The artifactual formation of a membrane capable of responding to valinomycin at the electrode tip can be ruled out by a variety of arguments:

1) The addition of valinomycin does not change the membrane potential when the mitochondria are in a medium approximately between 60 and 80 m*M* (*1*). This is consistent with an intramitochondrial $K^+$ concentration in that range and not with the 2*M* KCl contained in the microelectrodes. The alternative, that a pocket is formed at the mitochondrial membrane into which $K^+$ leaks from the electrode, is also unlikely. The potentials do not change significantly with time (up to 3 minutes) and are independent of the electrode taper and size of the tip (as measured from the electrode resistance) (*2*).

2) The results obtained with microelectrodes correspond quantitatively to those obtained with electrofluorimetric dyes in either *Drosophila* (*3*) or giant mitochondria of mice (*4*).

3) Finally, the same results are obtained whether the electrodes are filled with KCl or NaCl. Figure 1 shows the results of an experiment carried out with an electrode containing 2*M* NaCl. For six impalements the means (± standard deviation) were 16.8 ± 2.6 mv and −15.5 ± 2.8 mv in the absence and in


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