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Correspondence

Re: G. E. Moore, S. B. Merrick, L. K. Woods, and N. M. Arabasz. A Human Squamous Cell Carcinoma Cell Line. Cancer Res., *35:* 2684-2688, 1975.

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We find certain results and comments presented in the article by G. E. Moore *et al.* difficult to accept without further explanation. First (p. 2685), it is stated that "Assays for G6PD[1] mobility were performed from lysates of COLO 16 and HeLa in 0.9% NaCl solution giving negative results from COLO 16 and 5.4 IU for HeLa." Second (p. 2686), "Unfortunately, the G6PD isoenzyme mobility for type A variant that is characteristic for HeLa is not a critical test because COLO 16 was derived from a black female patient. However, no enzyme was detected in this cell line, in contrast to HeLa cells."

As to the first statement, one can only presume that neither electrophoretic mobility (a test that distinguishes qualitative electrophoretic variants) nor enzymatic activity exists for G6PD in these cells. This is a startling discovery. I have discussed this finding with three colleagues, all of whom are much better informed that I in enzymology of human cells, and this appears to be a totally unique case, as presented. Was mobility tested? How many samples were tested for activity?

The second statement, based solely on result(s) stated in the first, totally obfuscates innumerable studies relating to the electrophoretic mobility of isoenzymes of G6PD of different genotypes (G6PD type A, A, B, etc.), results of which have been used not only in medicine but also as a permanent and very reliable genetic markers of cells in long-term culture. Nevertheless, if the authors mean that the cells, with regard to G6PD mobility and/or activity, have not changed during 2 years in cultivation (a generally accepted concept) and thus reflect the patient's genotype, then her cells *in vivo* whether she was black or white would presumably not have shown any mobility or activity for G6PD either. This is irreconcilable with established biochemistry except, perhaps, in the remotest instance.

The authors have apparently confused activity of the enzyme, presumably in a kinetic assay for NADH coupling on the one hand with the mobility of the isoenzyme to an isoelectric point either slow (B) or fast (A) on the other. The latter mobility characterizes HeLa cells and many other cells or cell lines derived from a portion of the Negro population (1).

These results and the statements they engendered, if not more carefully explained, cannot but be misused or misinterpreted.

REFERENCE

1. Kirkham, H. N. Glucose-6-Phosphate Dehydrogenase. *In:* H. Harris and K. Hirschhorn (eds.), Advances in Human Genetics, Vol. 2, pp. 1-60. New York: Plenum Press, 1971.

[1] The abbreviation used is: G6PD, glucose-6-phosphate dehydrogenase.

We appreciate the opportunity to clear up the inconsistency regarding the G6PD activity in the cell line COLO 16, called to our attention by Walter A. Nelson-Rees. We have made the cell line available to him for further study.

He was quite correct in stating that the description of our assay of G6PD was confusing. We acknowledge the inadvertent error in the use of "mobility" when we meant "activity." A mobility assay was not done; only a test for G6PD activity was performed. There was no activity for G6PD in COLO 16 and thus one would assume that COLO 16 could not have positive mobility for an isoenzyme of G6PD. Hopefully, Dr. Nelson-Rees' studies will further clarify this point.

We deny that we implied that absence of G6PD activity in COLO 16 indicates an absence of activity in the patient's genotype. The assay for G6PD activity was included because of the import G6PD assays were given in distinguishing HeLa from other human tumor lines (1).

Lastly, we suppose a cell line derived from malignant tissue and shown to have an abnormal chromosome constitution may be positive or negative for any biochemical markers known for humans and may not necessarily reflect the donor's genotype.

REFERENCES

1. Nelson-Rees, W. A., Flandermeyer, R. R., and Hawthorne, P. K. Banded Marker Chromosomes as Indicators of Intraspecies Cellular Contamination. Science, *184:* 1093, 1974.

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Addendum

We have examined cells of a culture of COLO 16 kindly sent to us by Dr. Moore. The cells were found to have 45 to 48 chromosomes. All metaphases treated by trypsin-Giemsa for G banding revealed the 7q+ marker. We believe that it represents an addition of a portion of 12q to 7q. Other markers were also seen.

We did not observe chromosome markers characteristic of HeLa cells in the metaphases studied. These results confirm the findings of Moore *et al.* However, the cells as studied by Dr. W. D. Peterson, Jr., Child Research Center of Michigan, as well as by Dr. L. Hunter, of our laboratory, revealed type A (fast) mobility for G6PD. In this regard, they