A Permanent Heteroploid Human Cell Line with Type B Glucose-6-phosphate Dehydrogenase[1] (35455)

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Previously reported data (1–3) show that all permanent heteroploid human cell lines have type A glucose-6-phosphate-dehydrogenase (G-6-PD) isozyme, although many such lines originated from cells obtained from type B G-6-PD donors. One explanation of this finding is that all of these cell lines are contaminated by, and therefore are, substrains of the HeLa cell line (4). The latter was derived from cells of a presumed type A donor. An alternative explanation suggests that cells belonging to this classification have undergone similar modulative, epigenetic or genetic alterations *in vitro* as part of becoming established and perpetuated as permanent cell lines.

Additional evidence that favors one or the other explanation has been sought. Because truly early seed stocks of extant heteroploid cell lines were not available, it seemed worthwhile to initiate and study new lines.

This report describes the establishment of Det. 562, a permanent heteroploid cell line obtained from a type B G-6-PD donor. Cellular morphology and growth rate have altered during its culture for over 2 years, but after more than 120 passages *in vitro*, the cell line remains type B.

Methods and Materials. 400 ml of pleural fluid was removed from an adult Caucasian female with metastatic adenocarcinoma of the throat. After centrifugation, pelleted cells from the fluid were resuspended in growth medium consisting of minimum essential medium (Eagle) supplemented with sodium pyruvate, lactalbumin hydrolysate, and 20% fetal calf serum. 1×10^5 cells/ml of medium were inoculated into glass bottles and incubated at 37° in 5% CO_2–95% air atmosphere. Plastic bottles were used after the first four transfers. Frozen cell stocks were prepared by techniques previously described (5).

Cells were fed at 2–3-day intervals and monolayers were harvested by 0.2% trypsin. G-6-PD zymograms of cell extracts were made on sucrose agar-gel films, as previously described (2). Karyotypes of Det. 562 were made by well established procedures (6).

Results. When cells obtained from pleural fluid were inoculated into glass bottles and incubated, most of the inoculated cells failed to attach to glass. Those that did were predominantly elongated and fibroblast-like in appearance. By the end of the second week in culture, fibroblast-like cell growth was evident, but a number of epithelial-like cell foci were also seen. By the end of the fourth week, sufficient cells were present to permit transfer. Three additional transfers were made of these slowly growing cells. The outgrowth of transfer four was stored as an initial frozen seed stock.

From that frozen seed stock, subsequent sequential transfers were carried out that now exceed 120 passages. Figure 1A illustrates the morphologic appearance of the cell line in early passage (P12). The culture is shown to have a variety of cell types, mostly epithelial-like but also having giant and highly vacuolated cells. Cells in early transfer did not grow to confluency. With additional transfer, the appearance of the cell culture changed, as shown in Fig. 1B, which illustrates the appearance of cells at later transfer (P104). Cultural morphology is that of a confluent monolayer composed of typical polygonal epithelial-like cells that tend to crowd and pile up, with only an occasional multinucleated giant cell present.

Differences in the cell line between early and recent passage were also reflected in growth characteristics. The yield of cells per

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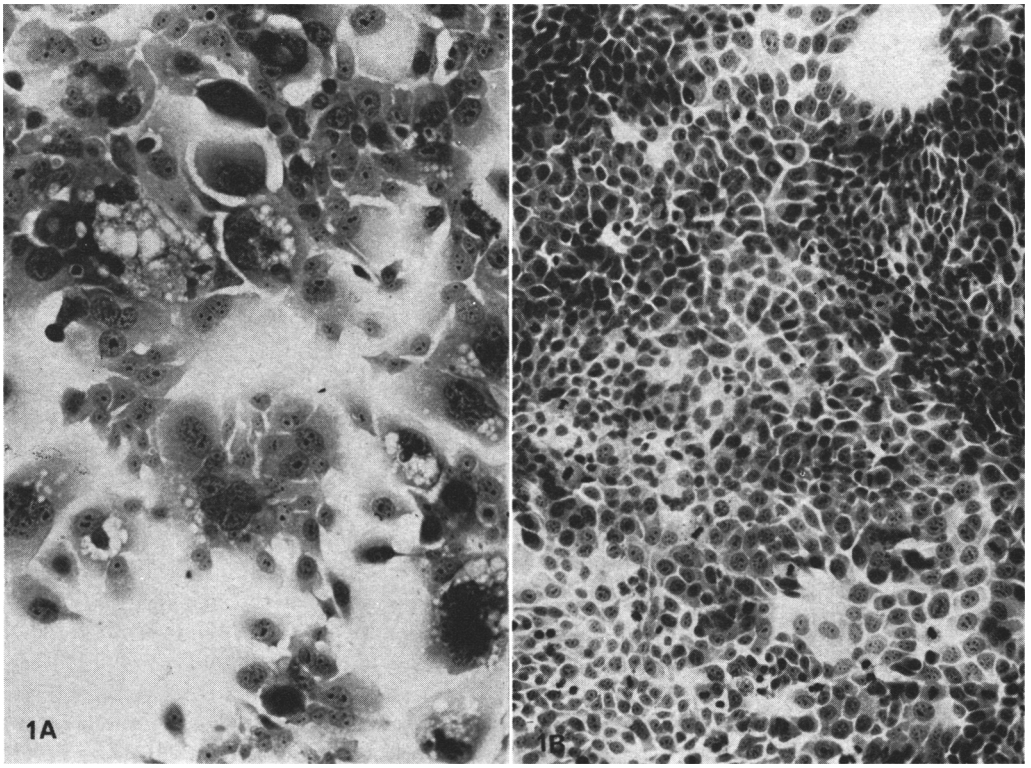

FIG. 1A. Morphology of Det. 562 at passage 12; (B) Morphology of Det. 562 at passage 104.

TABLE I. Increase in Cell Yield with Passage Level.

Passage no.	Av yield/flask (7 days)	Passage no.	Av yield/flask (7 days)
P1 –P10	1.1×10^6	P51–P60	7.6×10^6
P11–P20	2.2×10^6	P61–P70	13.3×10^6
P21–P30	3.9×10^6	P71–P80	16.5×10^6
P31–P40	4.3×10^6	P81–P90	17.3×10^6
P41–P50	9.5×10^6	P91–P100	16.8×10^6

flask increased with passage as the culture became established. As shown in Table I, average yields per plask per 7 days steadily increased with each 10 transfers. In late passages the yield is approximately 16 times that of earliest passages. The culture yield stabilized near the 70th passage level. The selection and adaptation of a predominate cell type in the culture appears to explain the pattern of increased growth yield followed by a leveling of yield in the latest passages.

Little variance in chromosomal findings was seen at different passage levels. As shown in Table II, the modal chromosome number of Det. 562 is 64–65, with a range from 53–71. Nearly 100% of examined cells have a

TABLE II. Chromosome Distribution of Det. 562 at Different Passages.

Passage no.	No. of cells examined	Modal no.	Range	% Polyploid (130±)	% With breaks fragments, etc.	% With markers
8	60	66	59–71	28	1.6	95–100
17	60	65	53–70	25	15	95–100
30	70	65	58–72	6	5.7	95–100
42	100	64	58–68	10	4	95–100
74	100	65	58–68	2	4	95–100
91	100	64	59–67	13	3	95–100

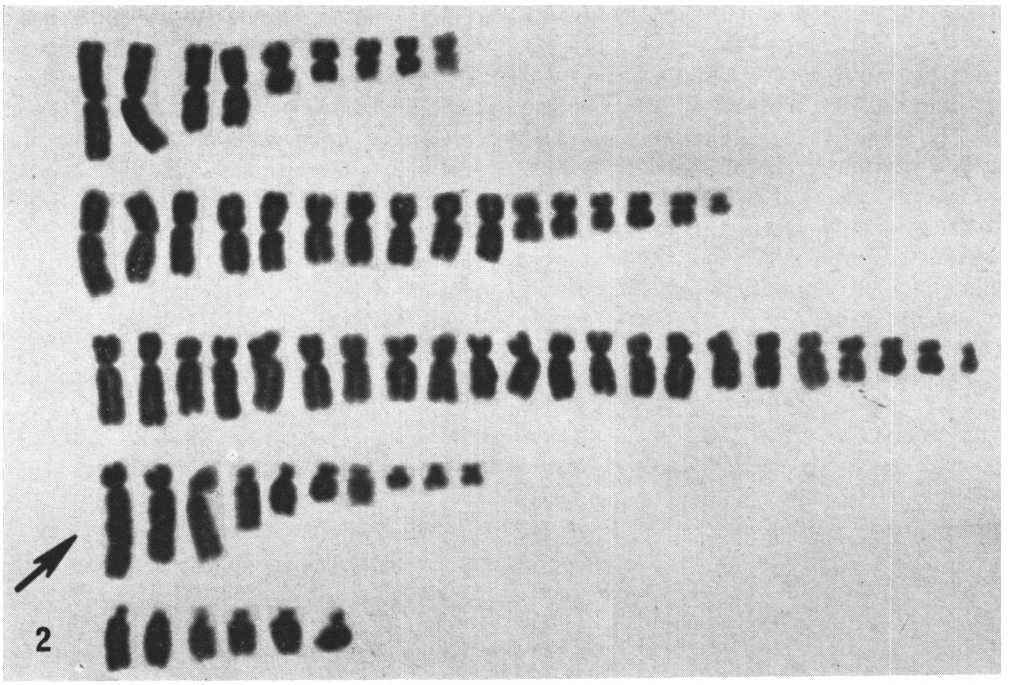

FIG. 2. Karyotype of Det. 562: The marker chromosome is designated by the arrow.

large subtelocentric marker chromosome useful for identifying the culture. Polyploid cells (130± chromosomes) were prominent in early passages, but less so in recent passage. About 40% of the cells also have minute markers. Figure 2 shows a cell karyotype with the chromosomes arranged by arm ratio and with the marker chromosome indicated. It is presumed that some selection occurred in cells to be examined in earlier transfers because of conditions chosen for karyologic examination. It is these cells that became predominant in the culture. No diploid or near-diploid cells could be found at any passage.

Qualitative examinations of extracts prepared from early and late transfer cells revealed no change in G-6-PD electrophoretic patterns. Figure 3 shows a zymogram of G-6-PD isozyme activity. Comparison can be made of mobilities of G-6-PD obtained from

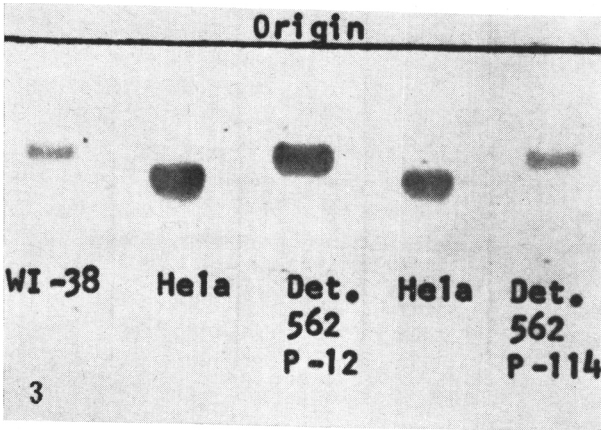

FIG. 3. Zymogram showing comparative mobilities of G-6-PD from WI-38, HeLa, and early and recent passage Det. 562 cells.

cultures of Det. 562 at P12 and P114 with those from HeLa and WI-38 cell lines. G-6-PD of both transfers of Det. 562 migrate at the same rate as that of WI-38 and are distinctly slower than that of HeLa. WI-38 has been previously characterized as type B and HeLa as type A G-6-PD (1–3). In addition, 20 colonies of Det. 562 at passage 77 were isolated, grown out, and extracts were prepared for electrophoresis. All 20 sublines were type B G-6-PD.

Det. 562 was also monitored periodically for susceptibility to viruses, and by determination of species antigens. The former included susceptibility to poliovirus, and the latter for human species surface antigens as revealed by specific immunofluorescence. Both tests were positive for these characteristics.

Discussion. In this report it has been shown that Det. 562 is a heteroploid human cell line, apparently permanent, sharing many of the characteristics of the prototype cell line, HeLa. It differs from most cell lines in its particular classification, in having type B G-6-PD isozyme.

The results indicate that long-term growth of heteroploid cell lines does not require type A G-6-PD, and confirm and extend the earlier observations made on tumor cells grown for shorter periods ot time by Gartler (1) and by Peterson *et al.* (2). Auersperg and Gartler (10) recently reported that a heteroploid tumor cell line, C-12R, irradiated 10 times in the course of 35 transfers *in vitro* also retained type B G-6-PD and phosphoglucomutase$_1$ 2-1 and PGM$_3$2.

These findings underscore the desirability of isozyme analysis for each newly established cell line as a means for determining the validity of that line. When a cell line possesses a G-6-PD type contrary to expectation, such as that recently reported by Fraley *et al.* (11), evidence to explain the discrepancy is required, since type A G-6-PD can no longer be considered characteristic of all permanent cell lines. Similarly, special properties of cell lines, such as morphology, virus susceptibility, or growth characteristics are insufficient markers by themselves of a new cell line, because these are properties that may be a part of the potential variation of cell populations already established. For example, the cell line isolated and reported by Sykes *et al.* (12) lacks description of important enzymic properties that would help indicate more clearly whether or not the line is new or an interesting variant that may have been derived from a previously existing cell line.

The establishment of a type B heteroploid cell line does not exclude the possibility that mutations affecting G-6-PD isozyme can have occurred in the past or can occur in the future. Certainly, the many variables inherent in culturing cells *in vitro* will influence different expressions of cell potentials. As one example, the medium utilized in growing Det. 562 differs from that in which earlier lines were originated. Mitchell *et al.* (7) have shown that neoplastic conversion of certain mouse cells *in vitro* is related in part to the species of serum incorporated in growth medium. Murphy *et al.* (8) showed that choice of serum altered growth and morphologic appearance of human cells *in vitro*. Hsu (9) reported that population structure of certain mouse cell lines is altered by modification in tissue culture techniques. Such differences in cell expression suggests that there may be as well an increased sensitivity of cells to mutational effects that is dependent upon conditions of growth.

It may be of significance that Det. 562 was cultured in a manner and medium different from that in which earlier lines originated. However, the irradiation of line C-12R (10) suggests that sensitivity to mutation of the G-6-PD allele would require substantially greater effect than that likely to be brought about by changes in tissue culture medium or technique. In the absence of evidence to the contrary, it is concluded that the information so far obtained favors Gartler's interpretation that most heteroploid cells are derivatives of HeLa.

Fundamental to an experimenatl design for measuring cell variation or mutational change is the capacity to adequately monitor cells. Cross contamination by cells of the same or different species continues to be a major problem in studying cell potentialities *in vitro*. Monitoring for genotypic characteristics provides a background against which

phenotypic expression of cell variation can be reliably evaluated.

Elements of a basic monitoring system are described in this report and include (a) freeze preservation of early and subsequent passage seed stocks at appropriate intervals; (b) identification of species and other antigens in cell populations; (c) study of appropriate enzyme activities; and (d) periodic examination of karyotype and frequency distributions.

Summary. Det. 562 is a permanent heteroploid human cell line that is similar to HeLa and other heteroploid cell lines in morphology, growth, and virus susceptibility.

It differs from similar permanent human cell lines in having G-6-PD type B isozyme. The implications of this finding are discussed. The importance of cell line monitoring is stressed.

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