PROPERTIES OF GALACTOSEMIC CELLS IN CULTURE

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Cell lines were developed from four millimeter skin biopsies on two patients with proven galactosemia, the heterozygous mother of one of these patients, and 5 non-galactosemic patients. In addition, cell lines were developed from sternal marrow aspirates on a galactosemic patient and another non-galactosemic patient.

Cultures were maintained in monolayer in Petri dishes and bottles. When a confluent monolayer was formed, the cells were trypsinized off the glass and a fraction of them was introduced into a fresh vessel. The fraction varied from one-half to one one-hundredth. A continuous record of the fractions by which each culture was split and the size of the surfaces it covered was kept. From these records the M-number, the approximate number of times the cells had increased as of a given moment, could be computed. Growth in the primary dish (the one with the explant) was ignored. When inadequate growth or other difficulties were encountered with a cell line, the line was often started again with another wave of cells from the primary dish (from which the explants were never removed). No difference between successive waves has thus far been found. However, a discrepancy between the age of the culture and the M-number results from this practice.

After at least two subcultures, the cells derived from skin were used in growth experiments: A large bottle of cells was subcultured into 20 to 100 smaller ones. Twenty-four hours later, after the cells had attached, and spread, they were washed with Eagle's (1959) minimum essential medium from which the hexose had been omitted. A random sample of 4 or 5 bottles was then taken to

determine the initial cell protein. The remaining bottles were divided into 5 groups and each was overlayed with one kind of experimental media. The experimental media consisted of 88% Eagle's (1959) minimum essential medium, from which hexose had been omitted, and 12% exhaustively dialyzed pooled human sera. Pyruvate and the "non-essential amino acids" specified by Eagle (1959) were each added in quantities to make a final concentration of 1 millimolar. Hexose was then added or withheld to make the 5 following experimental media with concentrations of 100 mgms % glucose, 100 mgms % galactose, 5 mgms % glucose, 95 mgms % galactose and 5 mgms % glucose (mixed hexose), and hexose-free. Each kind of medium was changed every 72 hours. Between 9 and 18 days after placing the cells in experimental media, the cell protein was again determined. Measurement of cell protein was by the method of Oyama and Eagle (1956). All values are based on the mean of 2 to 4 replicate bottles (usually 3). Two types of growth experiments were performed: In the "two-point" experiments only the initial cell protein and the cell protein at the end of the experiment were determined. In the "multiple point" experiments, cell proteins were determined on bottles removed from the experiment every 72 hours. In most of the two point experiments and all the multiple point experiments, galactosemic (or heterozygous) cells were grown concurrently with non-galactosemic cells, the two kinds of cells being fed with the same media.

The results of a multiple point experiment involving both galactosemic and normal lines are given in the two graphs in figure 1. Note that the galactose curve in the case of the non-galactosemic cells follows the glucose curve. In the galactosemic it follows the hexose-free curve.

In figure 2, the two-point experiments are summarized, growth ascribable to galactose is plotted against growth ascribable to glucose. The 9 day and final day results of the multiple point experiments are included in this graph. The aa line may not have quite a zero slope, perhaps due to minute quantities of contaminating glucose in the galactose.

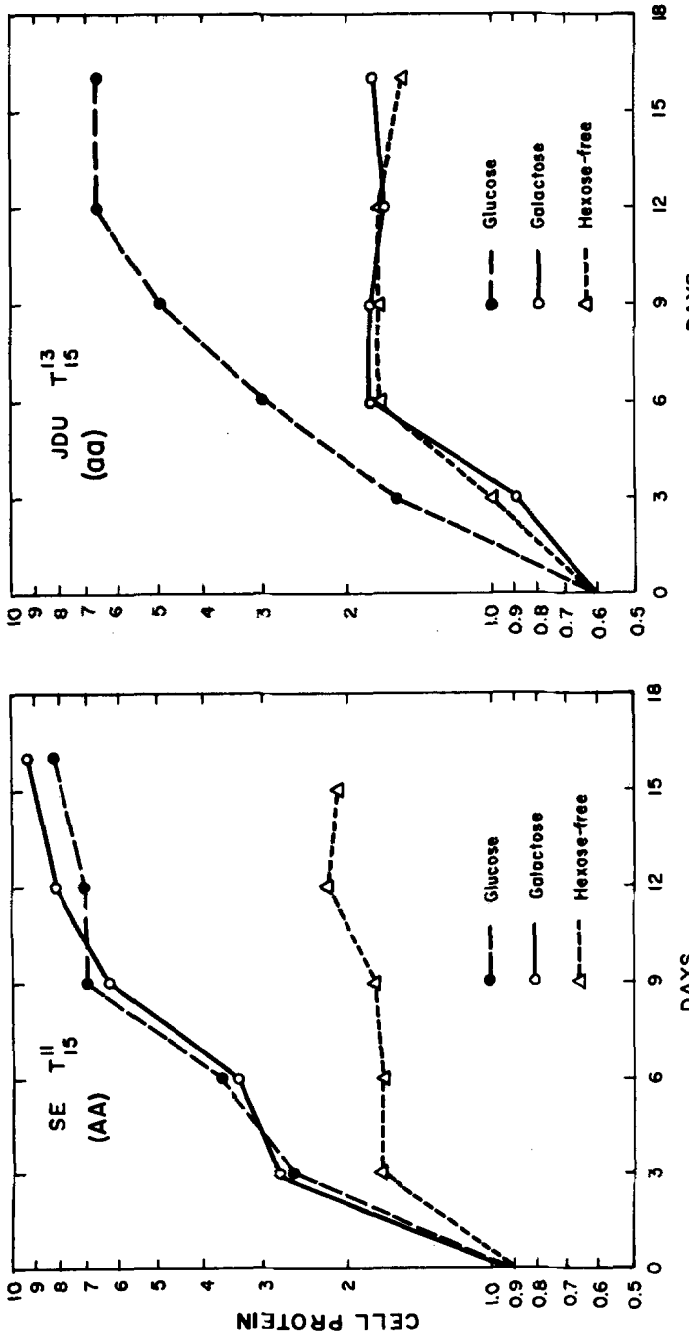

Figure 1. Cell growth from a galactosemic (aa) and a non-galactosemic patient (AA) in medium containing 100 mgms % glucose, 100 mgms % galactose and medium-free of hexose. In graphing, one unit of protein corresponds to 100 γ of bovine albumin. At the start of this experiment the galactosemic cells were 138 days old, had been sub-cultured 12 times, and had increased by more than 4.8×10^5. The non-galactosemic cells were 91 days old, had been sub-cultured 10 times and had increased by more than 6×10^4.

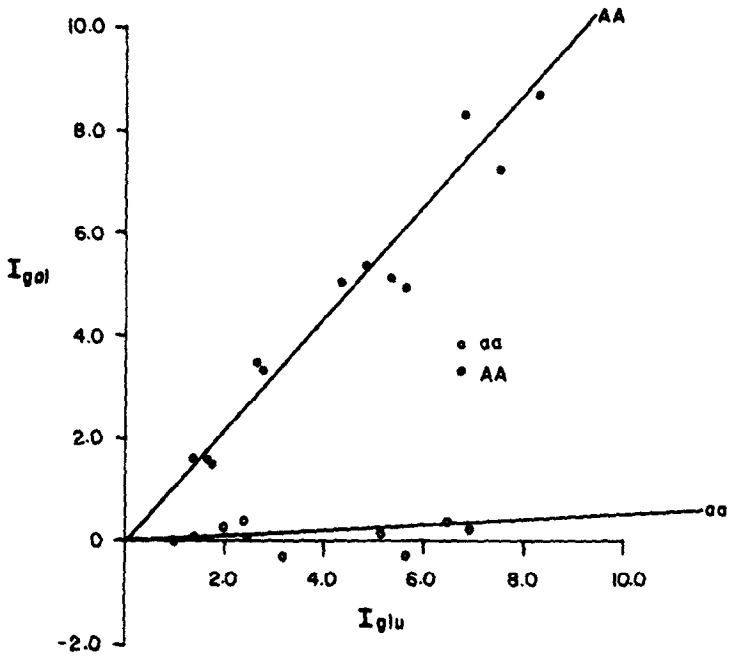

Figure 2. Growth of galactosemic (aa) and non-galactosemic (AA) cells in glucose and galactose: Two point experiments. I_{gal} is the growth (final cell protein) in medium containing 100 mgms % galactose minus the growth in hexose-free medium, divided by the initial protein. I_{glu} is the corresponding function for medium containing 100 mgms % galactose. The indices measure growth ascribable to galactose and glucose respectively.

The range of some of the parameters describing the two-point experiments are as follows:

Presumed Genotype	Range in ages* (days)	Range in no.* of previous sub-cultures	Range in* M-Nos
AA	48 to 149	2 to 19	4 to (4×10^{12})
aa	46 to 138	4 to 12	12 to (4.8×10^{5})

*Computed as of the beginning of each experiment

The heterozygous line (MAD) has been more variable in its relative growth in glucose and galactose than the homozygous cell lines. In most of the experiments, however, the heterozygous line has grown equally well in the two sugars.

Experiments comparing growth in the mixture of 95 mgms % galactose and 5 mgms % glucose with growth in 5 mgms % glucose suggest that non-galactosemic cells grow equally well in both media, except at high cell densities, where growth is better in the medium with the greater total hexose. The heterozygous

line is like the normals in this respect. The galactosemic cells, however, generally grow better in medium containing 5 mgms % glucose than in medium containing the 95/5 mixture. Multiple point experiments suggest that growth is slowed by the added galactose for the first 72 hours (or for a shorter time) and thereafter the cells grow at the same rate in the two media. This pattern of galactose sensitivity in the presence of glucose is somewhat reminiscent of the pattern first reported for transferase mutants of E. coli by Kurahashi and his colleagues (Kurahashi 1957, Kalckar et al 1957). However, among our human cells the effect is much less pronounced and can be made statistically significant only by pooling several experiments.

In the metabolic experiments, aliquots of cells were incubated with glucose-1-C^{14} or galactose-1-C^{14}, and the activity of the CO_2 produced was determined. The techniques were those of Weinberg and Segal (1960) for measuring the hexose metabolism of white blood cells.

The results of these experiments are given in Table 1. The variation in counts per million cells is probably due to our crude method of enumerating cells, which is accurate to but a factor of 2. The ratio R of counts from galactose to counts from glucose is less variable and appears to reflect genotype. The galactosemic cells are virtually unable to oxidize galactose.

Cells from several of these lines have been given to Dr. Herman M. Kalckar for enzymatic studies which will subsequently be reported.

Table 1

Isotope Experiments

	Line	"Age" (Days)	M-Number	Tissue of Origin	Millions of Cells Counted	(1) C^{14}gal counts per 10^6 cells per minute	(2) C^{14}glu counts per 10^6 cells per minute	R (1)/(2)
	(AA) MI^4	27	16	Marrow	7.8	220	380	0.6
	(aa) JDU^4	27	10	Marrow	3.9	0	870	0
AA	(AA) Be^7	65	8×10^3	Skin	4.3	352	807	0.4
	(AA) RCu^4	79	32	Skin	7.7	87	298	0.3
	(AA) Be^{11}	72	2×10^5	Skin	8.3	157	529	0.3
	(AA) Be^{20}	160	3.8×10^{12}	Skin*	1 Blake bottle	1235	3544	0.3
	(AA) SE^8	71	9.6×10^3	Skin*	1 Blake bottle	266	944	0.3
aa	(aa) BY^4	56	10	Skin	6.3	0	-	(0)
	(aa) JDU^6	55	240	Skin	5.3	7	590	0.01
	(aa) JDU^9	119	9.6×10^4	Skin*	1 Blake bottle	0	2644	0
Aa	(Aa) MAD^4	68	40	Skin	1.5	67	720	0.09
	(Aa) $MAD^{(5+6)}$	76	128	Skin	2.8	66	735	0.09

*Counts entered here are counts per minute per 1/3.2 of a Blake bottle.

AA denotes non-galactosemic patients, Aa the presumably heterozygous mother of galactosemic patient JDU and aa denotes galactosemic patients. The superscript on the letters designating the line is one plus the number of sub-cultures. Age is the time between the date of biopsy and the date of the experiment. Each unique combination of letters represents a different patient.

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