

STUDIES ON CELL LINES DEVELOPED FROM THE TISSUES OF
PATIENTS WITH GALACTOSEMIA*

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The value of studying inherited metabolic abnormalities in cell lines developed from the tissues of affected patients has been emphasized by Bööck and Kostman (1956), and more recently by Kalckar (1959a) and Luria (1959), among others. Such cell lines may eventually prove useful for the study of genetic changes such as back mutation, recombination, and transformation. In addition, they should occasionally yield fresh information about the nature of the disease itself.

One consideration, however, sharply limits the usefulness of cell culture in the study of genetically determined metabolic abnormalities. Cells which propagate for long periods in culture usually retain few, if any, of the specialized features of their tissue of origin (see Levintow and Eagle, 1960). Thus, a "liver" or "muscle" culture is not generally a collection of cells which can be shown to resemble liver or muscle. They are merely the cells which grew out when the specialized tissue was cultured.

The reason that cultured cells do not in general resemble their tissue of origin is unknown. It does seem clear, however, that if one wishes at present to study a genetic disease among actively growing cells, the disease must fulfill at least one requirement: the abnormality should be demonstrable in most, if not all, of the tissues of the body. That is, the disease must not be one where the function deranged is peculiar to some single tissue, for that function may well "disappear" in cultures of cells from normal as well as affected donors.

The disease galactosemia appears to fulfill the requirement. The enzyme, galactose-1-phosphate uridylyl transferase has been found in all normal tissues where it has been looked for. In the corresponding tissues of galactosemic patients, activity of the enzyme cannot be demonstrated (Kalckar, 1959a). The disease is due to a recessive gene with heterozygotes having enzyme activities about one-half normal (Kirkman and Bynum, 1959).

* Some of these investigations have been summarized in a preliminary note published elsewhere (Krooth and Weinberg, 1960).

In the present paper, growth and metabolic studies are described using cell lines developed from the tissues of galactosemics, non-galactosemics, and a presumed heterozygote. Enzymatic studies upon these same cell lines will be reported (Bias *et al.*, 1961).

Materials

Clinical Data.—

A summary on the patients used in these studies is given in Table I. It should be noted that the three patients with congenital anomalies have been karyotyped and found euploid.¹ The presumably heterozygous patient was the mother of patient JDU. Brief clinical descriptions of the two galactosemic patients are as follows:

TABLE I
Clinical Data

Patient (line)	Age of patient	Sex	Tissue biopsied	Diagnosis
RCU	4½ mos.	Female	Skin	Multiple malformations
BY	28 yrs.	Male	"	Galactosemia
BE	21 "	Female	"	Primary amyloidosis
JDU	11 "	"	"	Galactosemia
JDU (R)	11 "	"	Marrow	"
MAD	38 "	"	Skin	Galactosemia heterozygote (Mother of JDU)
SK	3 "	"	"	Multiple malformations
WIM	2½ "	Male	"	"
SE	20 "	"	"	Wilson's disease
MI	27 "	"	Marrow	Psoriasis and psoriatic arthritis

BY: 28 Year Old Male: After an uneventful delivery and neonatal period this patient developed milk intolerance, nausea, vomiting, and failed to gain weight. At 3 months of age he was hospitalized and found to have malnutrition, anemia, hepatosplenomegaly, albuminuria, and a urinary reducing substance which was later identified as galactose. On the institution of a galactose-free diet the melituria and albuminuria cleared, the patient gained weight rapidly, and became symptom-free. Except for the development of cataracts and mild mental retardation he has remained essentially well, though a restriction of galactose was never totally enforced. The patient has a negative family history for galactosemia, and no history of parental consanguinity.

The diagnosis of galactosemia has been confirmed by assays for galactose-1-phosphate uridyl transferase in red blood cells and liver (Anderson *et al.*, 1957).

JDU: 11 Year Old Female: The patient was born into a family (originally reported by Goldbloom and Brickman, 1946) with two older siblings, both of whom were affected with galactosemia. Galactose was omitted from her diet from birth, although it is uncertain how strictly this regimen was enforced. While the patient has never had the clinical symptoms or

¹ Karyotyping was carried out in collaboration with Dr. Hin Tjio, and the technique and detailed analysis will be published later.

signs of galactosemia, she has an abnormal galactose tolerance test, frequent urines with reducing substance, and a hemolysate of her red blood cells is unable to oxidize galactose- I - C^{14} to $C^{14}O_2$.

Composition of Media and Solutions.—

A. Growth medium:

	<i>By volume</i>
Pooled whole human sera ²	12 per cent
NCTC109 (McQuilkin <i>et al.</i> , 1957)	5 " "
Minimum essential medium (Eagle, 1959)	83 " "
<i>Supplement</i>	<i>Final concentration</i>
Pyruvate	1 millimolar
"Non-essential"	
Amino acids each at	1 millimolar
(Alanine, proline, serine, glycine, glutamic acid, asparagine, and aspartic acid)	

B. Experimental Media:

	<i>By volume</i>
Dialyzed pooled human sera	12 per cent
Hexose-free minimum essential medium	88 " "

The supplements are identical with those used for the growth medium, except that hexose is added as a supplement. The kind and final concentration of added hexose will be described subsequently. Sodium pyruvate was kindly prepared for us by Dr. Leon Levintow (Levintow and Price, 1952, Montgomery and Webb, 1954). No commercial pyruvate was used.

Trypsin Solutions.

Trypsin (1:300, Nutritional Biochemicals, Cleveland) was dissolved at concentrations of 0.05 and 0.25 per cent in a solution described by Puck *et al.* (1958), and is 40 per cent medium N 15 and 60 per cent saline G in their notation. 1 per cent of antibiotic solution (Puck *et al.*) was added.

Conditions of Dialysis.—

Pooled whole human serum, free of hemoglobin, is dialyzed 1 liter at a time for 21 hour⁵ against running tap water and then 3 hours against running distilled water, at about 5°C. The rate of flow is 425 ml./minute. The dialysis bag consists of cellulose casing (The Visking Corporation, Chicago) with an inflated diameter of 30/32 inches. The bag is mounted on a rocking table throughout the whole of the dialysis. Following the dialysis, the serum is free of glucose when tested by the method discussed by Chang (1960) using glucose oxidase on paper sticks (climistix, Ames Co., Inc., Elkhart, Indiana).

Source of Hexose.—

D-glucose and/or D-galactose (both Nutritional Biochemicals) were added to the experimental media. The galactose was first recrystallized from 70 per cent ethanol (after the method used by Kalckar *et al.*, 1959b) to remove contaminating glucose. Galactose- I - C^{14} (specific activity 4.72 μ c. per mg.) was obtained from the National Bureau of Standards and was chromatographically pure. Glucose- I - C^{14} (specific activity 6.74 μ c. per mg.) was obtained from the same source.

² For cell lines developed from bone marrow, 40 per cent type A₁ B single donor human serum is used; the other constituents of the growth medium are the same.

Methods

Development of the Cell Line From the Biopsy:

A technique for developing permanent euploid cell lines from human biopsies was published by Puck, Ciecura, and Robinson in 1958. Although our methods differ somewhat from theirs, the principles are the same.

A. Skin Biopsies.—The area biopsied in every case was the lateral surface of the shoulder, at about the level of the neck of the humerus. The skin was thoroughly scrubbed with phiso-hex,³ using a surgical brush, and then prepared first with zephirin³ and finally with 70 per cent ethanol. As much friction as possible was used in the preparation to ensure absolute sterility. 1 per cent procaine or xylocaine was infused intradermally and then subcutaneously, and a disc of skin 4 mm. in diameter was cut with a skin punch. Care was taken to include dermis, and subcutaneous fat was always visible when the disc was lifted up and its pedicle cut.

The tissue was placed in a dish, cut into 5 to 8 pieces with newly sharpened conjunctival scissors, and the pieces then partially minced. 5 ml. of 0.25 per cent trypsin solution were added, and the suspension of explants was transferred to a 25 ml. Erlenmeyer flask. 5 to 10 additional ml. of trypsin were added. The flask was stoppered and placed in a water bath shaker, adjusted to 37.5°C. Following agitation for 25 minutes, the suspension (now consisting of explants and cells) was transferred to a centrifuge tube and spun at 500 to 1500 R.P.M. for 10 minutes. The supernatant trypsin was removed, and the explants and cells were re-suspended in 6 ml. of growth medium. The suspension was divided among 3 Petri dishes (2 inches in diameter) and the dishes were incubated in 5 per cent CO₂ (saturated with water vapor) at 37.5°C. At 48 to 72 hours, the medium was removed (care being taken to leave the explants in the dish) and 0.60 to 0.75 ml. of fresh medium was added to each dish. Thereafter, this volume of medium was used and was changed every 48 hours. Large quantities of medium (of the order of 4 ml.) appeared to prevent growth when working with these minute quantities of tissue. In 4 or 5 days, individual "fibroblasts" (fusiform, transparent cells) could be seen attached to the glass, mainly in the immediate vicinity of the explants, from which most of them appeared to migrate. Epithelial outgrowths occurred from a number of explants. The epithelial monolayers eventually disappeared, and no epithelial cells were noted after trypsinization for the first subculture. Fibroblastic and epithelial outgrowths are shown in Fig. 1. This finding is consistent with that reported by Puck *et al.*, 1957.

15 to 25 days after the biopsy, from 10 to 20 per cent of the surface of 2 or 3 primary dishes was covered with one or more colonies of confluent cells; 1 dish was then subcultured with 0.25 per cent trypsin solution, the explants being left in the original dish. After decanting the trypsin suspension, the explants were reincubated with growth medium.

B. Bone Marrow Propagation.—Sternal marrow aspirates were obtained by the usual technique. The aspirate was collected in 5 ml. of a solution of 40 per cent N 15 (Puck *et al.*, 1958) and 60 per cent of their saline G to which 1 per cent of their antibiotic solution and several drops of 100 mg./ml. aqueous heparin had been added. The suspension was centrifuged for 10 minutes at 500 to 1500 R.P.M. and the fat floating on top of the supernatant and the buffy coat were inoculated into a total of 3 to 6 small tissue culture flasks (T₁₅), each containing 3 ml. of marrow growth medium. Thereafter, except for the serum composition of the medium (see Materials) the cultures were handled like those developed from skin. It should be noted that vast numbers of recognizable marrow elements attached to glass (and may even

³ Winthrop-Stearns, Inc., Rensselaer, New York.

have proliferated) in the primary bottles, but these did not come over on subculture. Here, as with skin, experiments were performed with the "fibroblastic" cells which eventually predominated. Marrow fat and the buffy coat appeared to yield these cells about equally.

Subculturing; Propagation; Computation of M-Numbers:

Cells were incubated with trypsin solution (in an air atmosphere) at 37.5°C. for 15 minutes to digest them off the glass. 0.25 per cent trypsin was used for the first subculture and 0.05 per cent trypsin for the subsequent ones. The trypsin suspension was centrifuged, the supernatant removed, and the cells resuspended in growth medium for reinoculation. Following the first subculture, larger volumes of media were employed. At each feeding about 0.2 ml of media were used for every square centimeter of surface area available for cell growth in the containing vessel.

Cells were at all times maintained as monolayers in Petri dishes and bottles, and after the first subculture were fed every 72 hours. When the cells of the layer became confluent, a fraction of them was subcultured into a fresh vessel. The fraction varied from $\frac{1}{2}$ to $\frac{1}{100}$. A continuous record of the fraction by which each culture was split and the size of the surface it covered was kept. From these records the M-number, the minimum number of times the cells had increased as of a given moment, could be computed. All "growth" of cells in the primary dish, the one containing the explant, was assumed to be exfoliation, and growth was not counted until the first subculture. Almost certainly we thereby underestimated cell growth.

After subculturing from the primary dish, the explants were fed until a new monolayer was generated—the new cells apparently coming largely from a fringe of the previous monolayer adhering to the explant. When inadequate growth or other difficulties were encountered with a cell line the line was started again with another wave of cells from the primary dish. In computing M-numbers growth in the primary dish was still ignored. We have thus far found no differences between successive samples of cells obtained in this way. However a discrepancy between the M-number and the age of the culture results from this practice.

Experimental Techniques:

Growth Experiments.—After at least two subcultures, the cells derived from skin were used in growth experiments: a large flask (a T₆₀ or a Blake bottle) of cells was subcultured into 20 to 100 smaller flasks (T₁₆'s). 24 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 group was overlaid with experimental media containing a different concentration of hexose. The five concentrations of hexose used were:

- 100 mg. per cent glucose
- 100 mg. per cent galactose
- 5 mg. per cent glucose
- 95 mg. per cent galactose and 5 mg. per cent glucose (mixed hexose)
- Hexose—free

Cells were not exposed to galactose prior to the experiments. 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). Cell protein is expressed in terms of the amount of bovine serum albumen giving the same optical density with the reagents used. (In the graphs and tables one unit of protein corresponds to 100 μ g. of bovine albumen. All values are based on the mean of 2 to 4 replicate bottles—usually 3).

TABLE II
Growth Experiments with Non-Galactosemic Cells (AA)

Line	BE ¹¹	RCU ³	BE ¹²	SK ⁶	RCU ¹¹	BE ¹⁴	SK ^{7,*}	SK ^{7,*}	WIM ⁸	BE ²⁰	SE ⁸	SE ^{11,*}	SE ^{11,*}
Duration, days.....	9	9	9	9	9	9	9	15	18	14	13	9	16
Initial protein.....	1.0	1.0	1.1	2.1	0.8	0.8	0.8	0.8	0.8	1.0	1.1	0.9	0.9
100 Glu.....	5.2	3.4	5.5	8.0	2.2	4.5	2.4	7.4	9.4	7.3	7.5	7.0	8.3
100 Gal.....	6.1	3.2	—	9.4	2.5	5.1	2.4	7.1	9.0	7.1	8.0	6.3	9.7
95/5.....	5.5	4.4	7.6	9.5	2.7	5.0	3.2	9.6	11.6	8.0	10.5	6.5	9.4
5 Glu.....	6.9	3.2	6.7	7.0	2.3	5.5	2.6	7.2	—	8.5	9.0	6.6	8.2
Hexose-free	2.5	1.6	1.8	2.2	1.1	1.0	1.0	1.3	2.3	1.9	2.1	1.8	2.1
"Age", days.....	79	86	86	44	107	100	60	63	61	163	84	100	107
M-No.....	1.7 × 10 ⁵	8 × 10 ⁸	5 × 10 ⁵	15	8 × 10 ⁵	6.4 × 10 ⁵	290	290	4	4.1 × 10 ¹²	8 × 10 ⁸	6.4 × 10 ⁴	6.4 × 10 ⁴

100 Glu and 100 Gal refer to media containing respectively 100 mg. per cent glucose and 100 mg. per cent galactose. 5 Glu and 95/5 refer to media containing respectively 5 mg. per cent glucose and the mixture of 95 mg. per cent galactose and 5 mg. per cent glucose. The superscript on the letters designating the line is 1 plus the number of subcultures. Each unique combination of letters represents a different patient. Age is the time between the date of the biopsy and the date of the experiment.

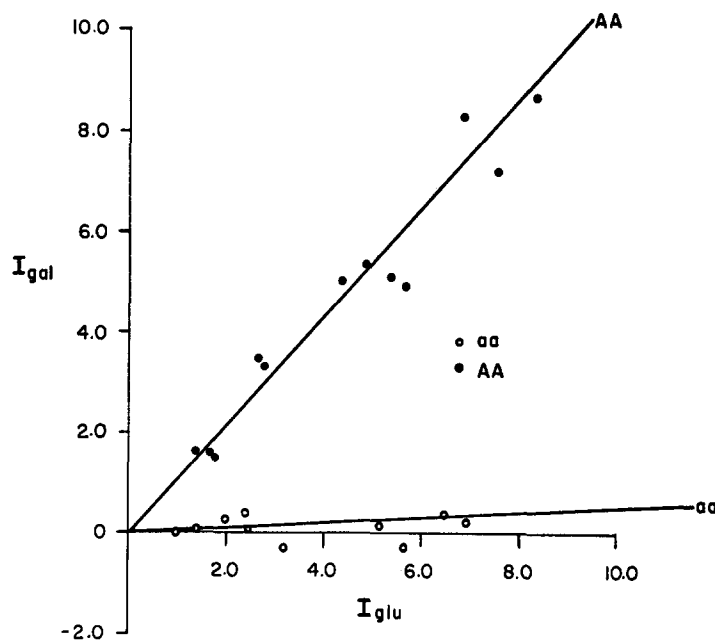
* Part of a multiple point experiment.

TABLE III
Growth Experiments with Galactosemic Cells (aa)

Line	JDU ⁶	BY ⁶	JDU ⁷	JDU ^{7,*}	BY ⁶	JDU ^{7,*}	JDU ⁸	JDU ¹⁰	JDU ^{12*}	JDU ^{12*}
Duration, days...	9	9	9	9	18	15	18	27	9	16
Initial protein...	1.3	0.9	0.7	0.6	0.8	0.6	0.9	0.6	0.6	0.6
100 Glu.....	5.2	3.3	2.0	1.5	3.9	2.2	8.5	4.9	4.9	5.7
100 Gal.....	2.0	1.5	1.1	0.9	1.1	1.2	3.0	1.4	1.8	1.8
95/5.....	5.3	3.0	1.6	1.2	2.7	1.7	8.7	3.5	3.3	3.9
5 Glu.....	5.9	3.4	2.0	1.7	3.4	2.2	—	4.5	3.8	4.8
Hexose-free.....	1.9	1.1	1.0	0.9	1.3	1.0	2.6	1.5	1.7	1.5
"Age" days.....	55	104	69	71	122	77	145	147	147	154
M-Nos.....	60	12	640	120	80	120	80	1.1 × 10 ⁴	4.8 × 10 ⁵	4.8 × 10 ⁵

See Table II for notation.

* Part of a multiple point experiment.



TEXT-FIG. 1

Two types of growth experiment were performed: in the "2-point" experiments only the initial cell protein and the cell protein at the end of the experiment was determined. In the "multiple point" experiments, cell proteins were determined on bottles removed every 72 hours. In most of the two point experiments and all the multiple point experiments, galactosemic cells were run concurrently with non-galactosemic cells, the two kinds of cells being fed with the same media.

Isotope Experiments.—Equal aliquots of cells were incubated with glucose-1-C¹⁴ or galactose-1-C¹⁴, and the activity of the C¹⁴O₂ produced was determined. The techniques were those of Weinberg and Segal (1960).

RESULTS

*Growth Experiments:**A. The Homozygous Lines.—*

Relative growth in glucose and galactose: Tables II and III contain the data from our two point experiments. Included also in the Tables are the data from the 9th and final day of those multiple point experiments in which the cells were grown in all 5 experimental media. Note that among the non-galactosemic cells (Table II) the growth in galactose is about equal to the growth in glucose. The galactosemic cells, however, grow better in glucose. The growth in galactose is about equal to the growth in medium which is hexose-free.

In Text-fig. 1, using the data from Tables II and III, growth ascribable to glucose is plotted against growth ascribable to galactose for galactosemic (*aa*) and non-galactosemic cells (*AA*). The index I_{glu} is defined:

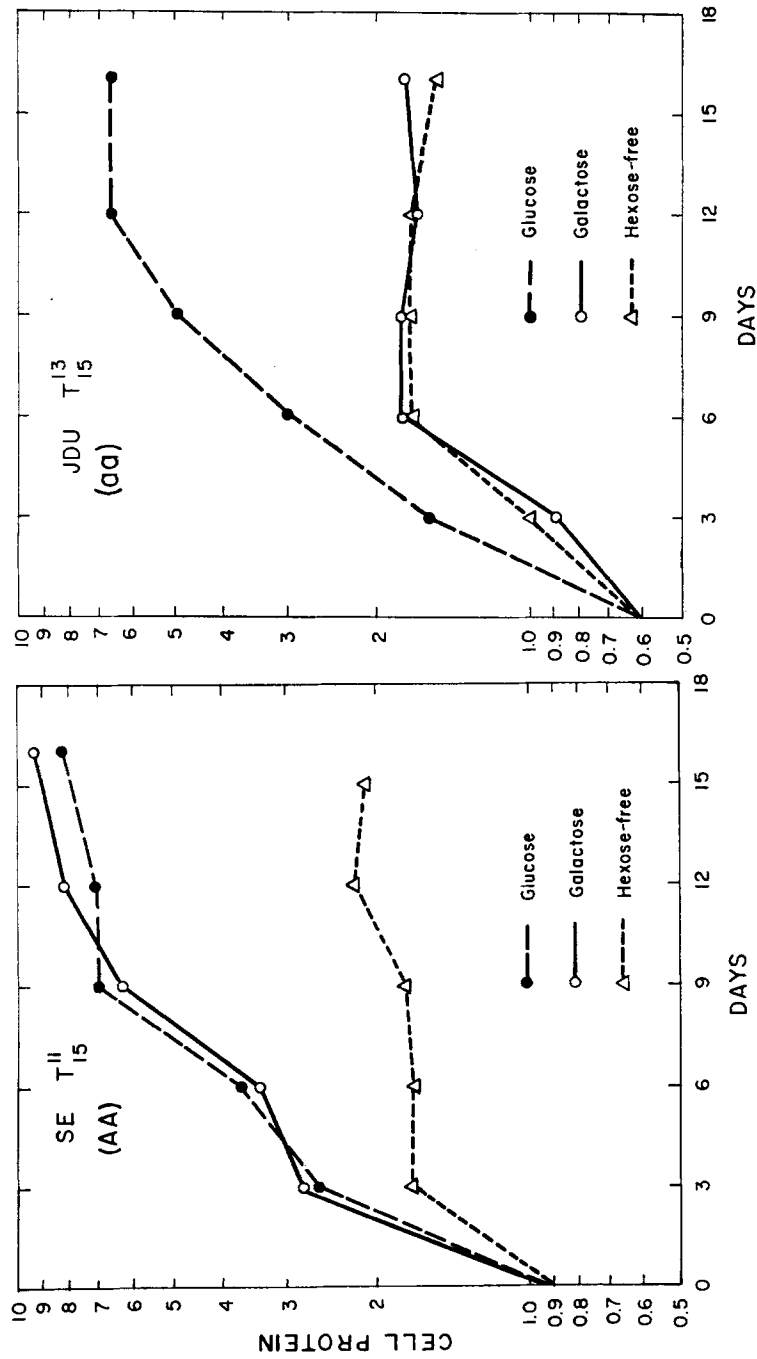
$$I_{glu} = \frac{\text{Growth (final cell protein) in medium containing 100 mg. per cent glucose minus growth in hexose-free medium}}{\text{Inoculum (initial cell protein)}}$$

I_{gal} is the corresponding function for galactose.

The two types of cells appear to differ sharply. The *aa* line may not have a zero slope, perhaps due to the presence of minute quantities of glucose in the galactose; glucose is known to persist as a contaminant of galactose even after two crystallizations in 70 per cent ethanol (Kalckar, 1960).

In Tables II and III, note the marked variation from experiment to experiment in the absolute growth rate. Some of this variation is probably spurious. A few of the experiments (*e.g.*, one JDU⁶ and one JDU¹⁰ in Table III) were deliberately allowed to run for a long period of time after the cells had built up to see if the galactosemic cells in galactose would ever tend to catch up with the ones in glucose. In addition, we have evidence that there is variation from experiment to experiment in the population density at which the cells plateau, a phenomenon which is easily confused with a slow growth rate in two point experiments. Beyond this, however, there is a true variation in the absolute parameter of growth from experiment to experiment. It is of interest that the differences in growth rate and in the density at which the cells plateau usually characterize the *experiment* rather than the line. Cells from an individual donor have not in most cases tended consistently to grow well or poorly. It is our impression that the variation we observe may be due chiefly to the character of the serum in the medium (Puck *et al.*, 1957), possibly involving immunological reactions between serum and cell, rather than to some intrinsic property of the cell. Superior and more uniform growth appears to result when pretested serum is employed in our hands as well as Puck's (1957). However, adequate screening has not always been possible.

In Text-fig. 2, data from a multiple point experiment are given. Both the



Text-Fig. 2

galactosemic and non-galactosemic cells grew well in this experiment. Note that the galactose curve in the case of the non-galactosemic cells follows the glucose curve, whereas in the galactosemic cells it winds about the hexose-free curve.

In Fig. 2, photographs are shown of galactosemic and non-galactosemic cells growing in the two sugars. The photographs are of replicate bottles and were taken at 9 days.

Galactose sensitivity: Growth in media containing 5 mg. per cent glucose was compared with growth in media containing the mixture of 95 mg. per cent galactose and 5 mg. per cent glucose. We wished to see if galactose sensitivity of the sort observed in the Gal-1-P uridyl⁴ transferase mutants of *Escherichia coli* (Kurahashi and Wahba, 1957 and 1958, Kalckar *et al.*, 1957) occurred among human galactosemic cells.

Our multiple point experiments when pooled yielded 14 observations in which mean growth was simultaneously recorded in the medium containing the mixture of 95 mg. per cent galactose and 5 mg. per cent glucose (95/5 medium) and the medium containing 5 mg. per cent glucose (0/5 media). The data⁵ are as follows:

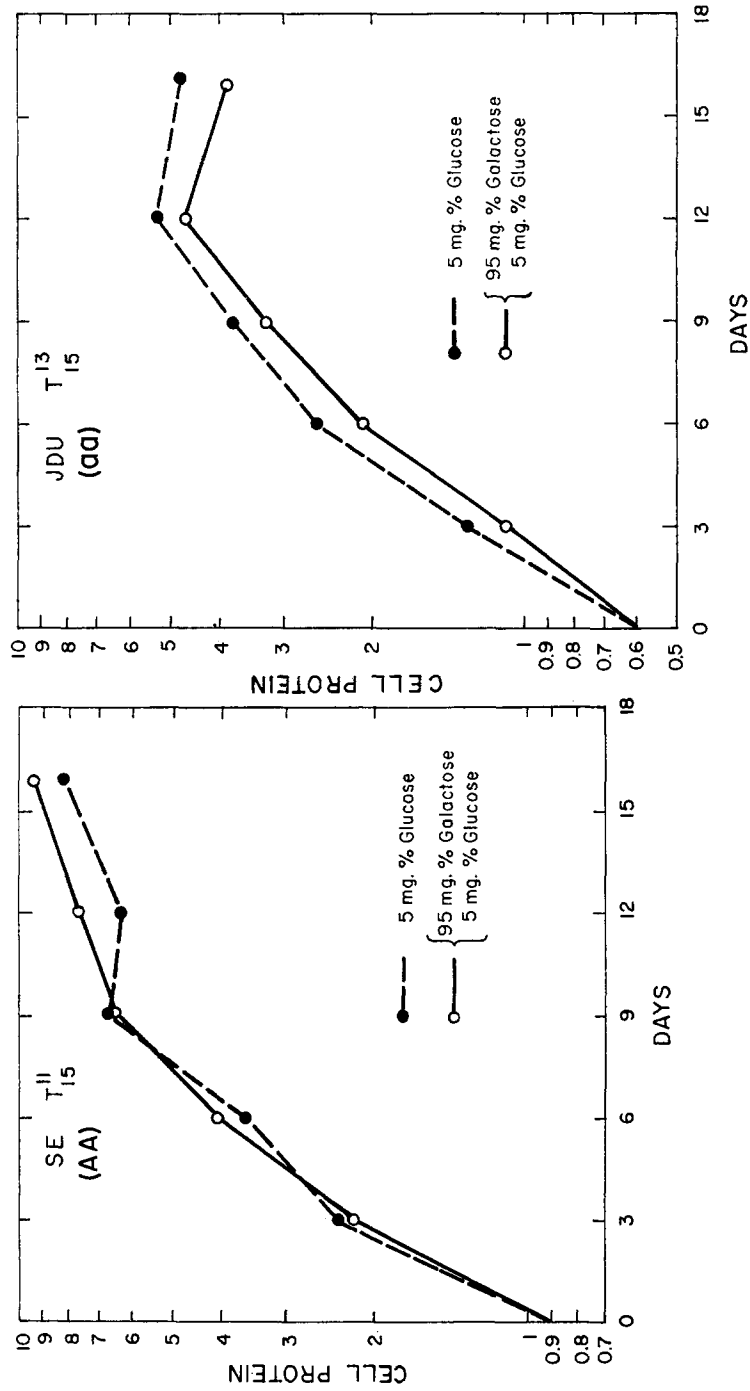
Presumed genotype	Growth in 95/5 greater than growth in 0/5	Growth in 0/5 greater than growth in 95/5	Growth equal	Total
<i>AA</i>	8	3	3	14
<i>aa</i>	1	11	2	14

It would appear from the data on the non-galactosemic cells that growth in the mixed media is, if anything, better than growth in 5 mg. per cent glucose alone. Among the galactosemic cells, however, growth in the mixed medium is usually poorer than growth in glucose alone. Perhaps the superior growth of the *AA* cells in 95/5 is due to the greater concentration of hexose which seems important at high population densities or perhaps to some synergistic combination of the two hexoses—we are not sure which. In either case one would not expect better growth in 95/5 among the *aa* cells since they appear to be unable to utilize galactose. However, inability to utilize galactose does not explain the fact that the *aa* cells do *less well* in the medium containing galactose. Therefore, these data at least suggest that the growth of galactosemic cells is inhibited by galactose.

The significance of these data can be tested by chi square, letting the assumption of equal growth in 95/5 and 0/5 generate the expected numbers. On this

⁴ Galactose-1-phosphate uridyl transferase.

⁵ Growth was considered greater in one medium than in another if the means of 2 to 4 replicates in the two differed by an amount of protein equivalent to at least 10 μ g. of bovine albumen.



TEXT-FIG. 3

assumption,⁶ there should be as many cases where growth is better in 95/5 as cases where growth is poorer, that is, 6 and 6, rather than 1 and 11. Chi square (using Yates' correction) is 6.8 for one degree freedom, which is significant.

In Text-fig. 3, a multiple point experiment is graphed. The effect is small. Note that the action of galactose appears to delay growth for the first 72 hours or less. Thereafter, the curves rise with equal slope. This type of an effect is similar to that reported by Kurahashi and Wahba (1957) in certain of their experiments where transferase mutants of *E. coli* were grown in a mixture of glucose and galactose. Our effect, considering the 19:1 ratio of galactose to glucose we use, is much smaller than the one they noted. However, they also noted that the galactose sensitivity could be abolished by the addition to the medium of 500 mg. per cent whole yeast extract. It is possible that the dialyzed serum in our experimental media is partially supplying the same unknown principle.

The two point experiments seem (to us) to be less suited for detecting a small difference in growth rate. The data are as follows:

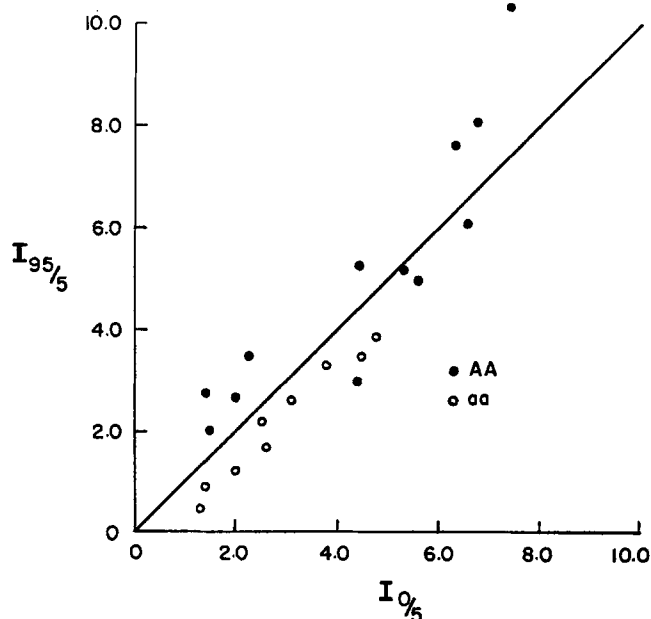
Presumed genotype	Growth in 95/5 greater than growth in 0/5	Growth in 0/5 greater than growth in 95/5	Equal growth	Total
<i>AA</i>	8	3	1	12
<i>aa</i>	0	5	0	5

The data therefore resemble the findings in the multiple point experiments. However, the 5 observations do not differ significantly from the expected values of 2.5 in each class on the null hypothesis. If one takes all the data in Table III (which includes 4 entries from multiple point experiments), there are 9 observations, with growth in 0/5 being greater than growth in 95/5 in every case. Summing the terms of a binomial expansion, a two-tailed probability of getting such data or a poorer fit on the null hypothesis is 1/256. The data on galactose sensitivity in Tables II and III are graphed in Text-fig. 4.

If, in Table III, one compares growth in media containing 100 mg. per cent glucose with growth in 95/5, we find better growth in the former case in 8 instances, and in the latter in 2, which is non-significant. Table III also reveals that the *aa* cells generally yield slightly less protein in hexose-free medium than in medium containing 100 mg. per cent galactose. This *prima facie* is

⁶ A non-parametric test rather than an analysis of variance of the actual protein values is used. Occasionally, 3 or 4 replicate bottles in the same medium will contain one value markedly different from the others. Sometimes the reason is discernible (contamination, inadequate washing of the bottle before use, etc.). Other times it is not. In both cases the extreme value is omitted. We are confident this practice gives us better estimates of the mean than would the inclusion of extreme observations. It deprives us, however, of the right to estimate the variance among replicate bottles.

inconsistent with a galactose sensitivity effect. We suspect that minute quantities of glucose contaminating our galactose may make the comparison unfair.



TEXT-FIG. 4

TABLE IV

Growth Experiments with the Heterozygous Line (Aa)

Line	MAD ⁶	MAD ⁸	MAD ⁷	MAD ¹⁰	MAD ⁵	MAD ⁴	MAD ¹⁴
Duration, <i>days</i>	9	18	13	35	32	28	9
Initial protein	0.7	0.9	1.1	0.6	0.9	0.7	1.1
100 Glu.	2.1	3.4	4.0	3.1	3.0	2.6	3.9
100 Gal.	1.5	2.3	3.8	1.4	3.0	2.3	3.9
95/5	2.2	3.2	4.0	2.4	2.7	2.5	—
5 Glu.	—	—	3.9	1.9	1.6	2.9	4.6
Hexose-free	1.2	1.7	1.8	1.4	1.5	1.4	1.6
"Age" (<i>days</i>)	76	93	139	153	155	151	195
M-Nos.	128	64	36	512	40	108	2.3 × 10 ⁴

See Table II for notation.

Certainly the inclusion of pyruvate and the non-essential amino acids (including alanine) in the medium renders the cells exquisitely sensitive to glucose (Chang and Geyer, 1957).

The Heterozygous Line.—Data on the growth of the heterozygous line are

given in Table IV. For some time marked technical problems were encountered with the handling of this line, and the absolute growth in most of the experiments has been intractably slow. Only two point experiments have thus far been done. However, all were terminated before the cell density reached the usual plateau levels. It is clear from Table IV that the heterozygous line was more variable than the homozygous ones in its relative growth in glucose and

TABLE V
Isotope Experiments

Line	"Age"	M-No.	Tissue of origin	Millions of cells counted	(1) C ¹⁴ O ₂ from C ¹⁴ gal: counts per 10 ⁶ cells per minute	(2) C ¹⁴ O ₂ from C ¹⁴ glu: counts per 10 ⁶ cells per minute	Ratio (1): (2)
	<i>days</i>						
(AA) MI ⁴	27	16	Marrow	7.8	220	380	0.6
(aa) JDU ⁴	27	10	"	3.9	0	870	0
(AA) Be ⁷	65	8 × 10 ⁸	Skin	4.3	352	807	0.4
(AA) RCU ⁴	79	32	"	7.7	87	298	0.3
AA (AA) BE ¹¹	72	2 × 10 ⁸	"	8.3	157	529	0.3
(AA) BE ²⁰	160	3.8 × 10 ¹²	"	1 Blake bottle	1235	3544	0.3
(AA) SE ⁸	71	9.6 × 10 ⁸	"	" "	266	944	0.3
(aa) By ⁴	56	10	Skin	6.3	0	—	(0)
aa (aa) JDU ⁶	55	240	"	5.3	7	590	0.01
(aa) JDU ⁹	119	9.6 × 10 ⁴	"	1 Blake bottle	0	2644	0
(aa) JDU ¹⁷	192	2.3 × 10 ¹⁰	"	1 " "	1	4541	0
Aa (Aa) MAD ⁴	68	40	Skin	1.5	67	720	0.09
(Aa) MAD ^(s+e)	76	128	"	2.8	66	735	0.09

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 1, plus the number of subcultures. Age is the time between the date of biopsy and the date of the experiment. Each unique combination of letters represents a different patient. Each flask contained of the order of 10⁶ cells, and 0.472 μ c. of either galactose-*J*-C¹⁴ or glucose-*J*-C¹⁴ (approximately 250,000 c.p.m.).

* Counts entered here are c.p.m. per 1/3.2 of a Blake bottle.

galactose. However, in three experiments, all terminated before plateau levels, growth in glucose and galactose were so close that a true difference appears unlikely. However, there must be factors influencing the growth of this cell line over which we still exercise imperfect control.

Isotope Experiments.—The results of the isotope experiments are given in Table V. Note that the ratio of counts as C¹⁴O₂, from galactose to counts from glucose appears to be fairly constant and to reflect genotype. The effect seems to persist over many months, and after the cells have increased many billion-fold. The variation within genotype of counts per million cells is probably due largely to our crude method of enumerating cells, which is accurate to but a factor of 2.

GENERAL REMARKS

In addition to galactosemia, a number of other diseases are probably susceptible to study in this way, *e.g.*, orotic aciduria (Huguley *et al.*, 1959, Smith and Huguley, 1960), acatalasemia (Takahara 1952, Takahara *et al.*, 1960, Wyngaarden, 1960), cystathioninuria (Harris *et al.*, 1959) and perhaps some of the other hexose and pentosurias (see Stanbury *et al.*, 1960, Rapaport, 1959, and Hsia, 1959). Indeed the number of possible diseases appears to be increasing sharply with time.

Cells of this kind are, as noted earlier, of potential use in the demonstration of genetic exchange. The possibility of transforming human cells has been made particularly attractive by Gartler's (1960) recent work suggesting that Earle's "L" cells will incorporate polymerized DNA *as such* from the medium into their nuclei. "Primary" human cells offer certain advantages. They are at present the sole source of mutant mammalian cells, where both the genetics and the identity of the abnormal protein (a particular enzyme in the present case) are known.

SUMMARY

Cell lines were developed from biopsies on galactosemic and non-galactosemic patients. It was shown that one can discriminate between lines from the two types of donors by their relative growth in glucose and galactose and by their ability to oxidize galactose- $I-C^{14}$. The latter method was successful in distinguishing a heterozygous cell line from the normal ones. Sensitivity of galactosemic cells to galactose was suggested by some of the experiments. The kinetics of growth were in some ways reminiscent of a similar phenomenon in the transferase mutants of *E. coli*, though in the human cells the effect was much less marked.

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EXPLANATION OF PLATES

PLATE 112

FIG. 1. Exfoliation from biopsy, SK line. $\times 150$.



(Krooth and Weinberg: Galactosemic cells in culture)

PLATE 113

FIG. 2: Growth in 100 mg. per cent glucose or galactose. Growth in galactose is shown in the two left hand panels and growth in glucose in the right hand ones. *AA* refers to the normal BE line and *aa* to the galactosemic JDU line. $\times 75$.

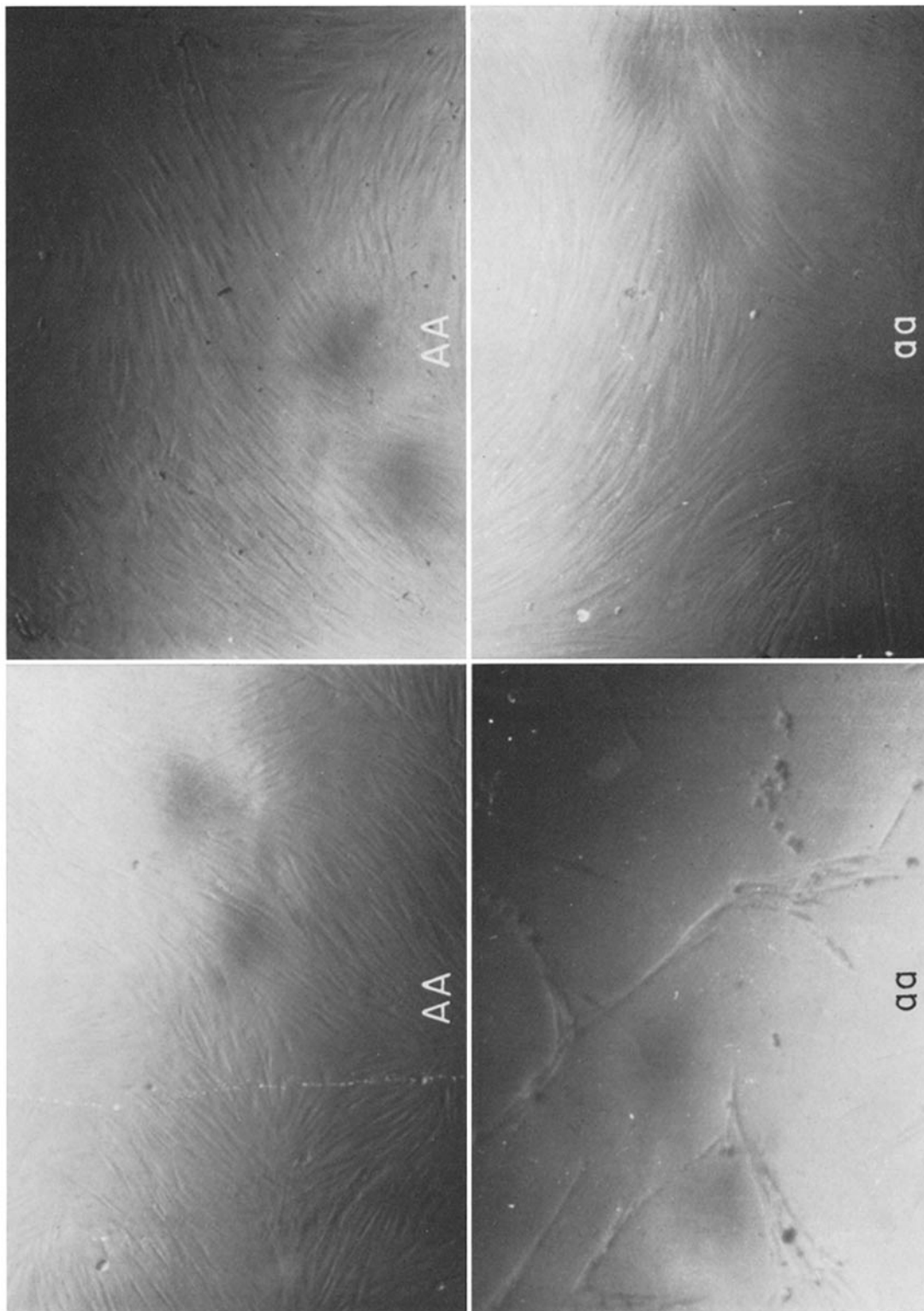


FIG. 2

(Krooth and Weinberg: Galactosemic cells in culture)