Biochemical Genetics 1:11–24 (1967)

UDP-Glucose: α-D-Galactose-1-Phosphate Uridylyltransferase Activity in Cultured Human Fibroblasts

James D. Russell and Robert DeMars

Received 23 Jan. 67—Final 14 Feb. 67

Galactose-1-phosphate uridyl transferase activity of normal, heterozygous and galactosemic strains is determined throughout the culture cycle of human fibroblasts using a new direct method of assay. The enzyme activities of high-density, stationary-phase cultures define three nonoverlapping classes, which correspond to the genotypes of the donors. During rapid growth, however, galactosemic strains show near-normal transferase activity. The incorporation of ^{14}C from $^{14}C_1$-galactose by living cells is measured. While heterozygous strains do not appear to differ from normal controls, homozygous mutant cells incorporate ^{14}C at about one-half the normal rate throughout the culture cycle. Variables affecting the assay are investigated and the implications of our results for further genetic studies of mutations affecting transferase are discussed.

INTRODUCTION

Galactosemia is a well documented inborn error of metabolism inherited as an autosomal recessive trait (Holzel and Komrower, 1955; Hugh-Jones *et al.*, 1960). The mutations are known to affect a single enzyme in the metabolic pathway of galactose (Kalckar *et al.*, 1956; Isselbacher *et al.*, 1956), UDP-glucose: α-D-galactose-1-phosphate uridylyltransferase (EC 2.7.7.12,[1] referred to as "transferase" below):

$$\text{GALACTOSE-1-P} + \text{UDP-GLUCOSE} \xrightarrow{\text{TRANSFERASE}} \text{UDP-GALACTOSE} + \text{GLUCOSE-1-P}$$

Measurement of transferase activity in red blood cells, by several methods, indicates that galactosemic individuals have little or no activity, while galactosemic hetero-

Paper #1105 from the Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin. This work was supported by the National Institutes of Health (Grants #GM-08217, #GM-398, and #GM-06983).

[1] Enzyme Nomenclature: *Recommendations (1964) of the International Union of Biochemistry* (1965), Elsevier Publishing Company.

zygotes have enzyme levels which are lower than those of normal persons. (Hsia *et al.*, 1958; Kirkman and Bynum, 1959; Donnell *et al.*, 1960; Bretthauer *et al.*, 1959). The UDP-glucose consumption assay (Anderson *et al.*, 1957) previously used for red cells has proved to be inapplicable to other cell types. To our knowledge, no sensitive and direct alternative procedure for cells other than erythrocytes has yet been described. The new method described here is both sensitive and direct and was devised with the expectation that it would further *in vitro* genetic studies of galactosemic cells. Our assay, like that of Ng *et al.* (1964), uses the substrate ^{14}C-galactose-1-phosphate. However, the products of the reaction are separated by a different method, since several years ago we found the charcoal separation method to be unsuitable in our system.

The quantitative transferase assay described here separates fibroblast cultures into three nonoverlapping classes, defined by near-zero, intermediate, and maximal specific activities, corresponding to the homozygous mutant, heterozygous, and normal genotypes of the fibroblast donors. Our experiments strongly suggest that homozygous mutant cells have significant transferase activity during rapid growth. Some of these experiments were performed under conditions that we later found to be suboptimal for the assay procedure, and extensive repetitions have been hampered by the unavailability of adequately pure substrate. However, tests of representative examples of the three genotypes with the optimal assay indicate that the results of the earlier experiments are, nevertheless, valid.

MATERIALS AND METHODS

Skin biopsies of diagnosed galactosemic homozygotes and heterozygotes were obtained by Dr. Frank A. Walker. The biopsies were prepared as previously described (DeMars and Nance, 1964), and the cells were propagated in medium F_4 supplemented with 15% fetal bovine serum according to the general procedures of Ham and Puck (1961). Control strains were taken from the laboratory collection.

Living cells were preserved by freezing at the second and third subcultures. The cells were suspended in a mixture of the medium and glycerin (9:1) and 1-ml aliquots of the suspension containing about 10^6 cells were sealed in glass ampules and cooled at a rate of 1 C/min in a Canalco liquid nitrogen freezer. The frozen ampules were stored under liquid nitrogen. When needed, the suspensions were thawed rapidly at 37 C and diluted 1 to 10 with fresh medium. The thawed cells were propagated in the same way as the original cultures. Cells were grown for experiments either in macro- or microcultures.

Macrocultures. Cells were grown in 60 mm diameter dishes, which hold about three million cells when full. The cells were prepared for enzyme and protein determinations by washing with 0.9% NaCl, scraping from the dish with a rubber policeman, centrifuging, suspending 5×10^6 cells/ml in water or buffer, and sonicating in a Medtron sonicator for 1 min.

Microcultures. Five thousand cells, in 0.2 ml of medium, were planted in microculture vials. These vials were made by gluing 9 mm diameter pyrex cylinders to microscope slides with clear silicone adhesive (both brands tested—Dow-Corning and

General Electric—are suitable). A twentyfold increase in cell number could usually be achieved in one week by maintaining rapid growth with daily renewals of culture medium. The micromethod clearly reduced the cost and space requirements of huge-scale experiments like those depicted in Fig. 7. This method also eliminates much handling of the cells. The monolayers were washed with 0.9% NaCl and fixed with acetone (4 C) for 1 min. Transferase activity and total cell protein can be assayed directly in the culture vessels.

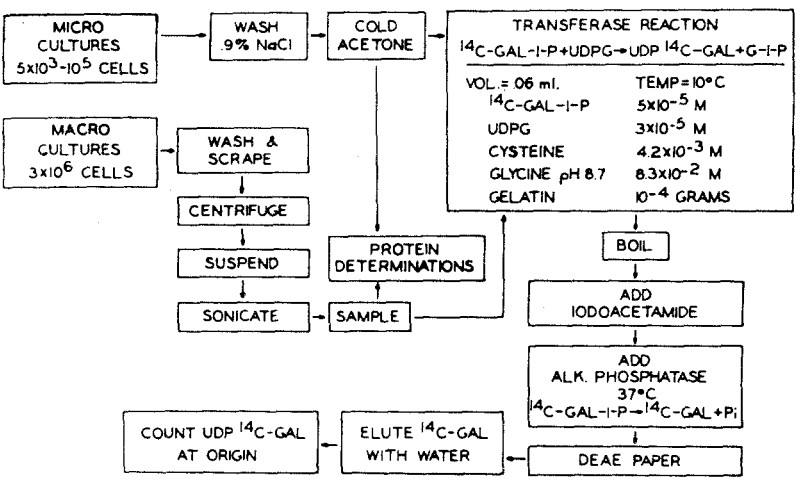

Fig. 1. Outline of the transferase determination.

Total cell protein was determined by the method of Oyama and Eagle (1956). Reagent volumes were reduced by a factor of one-fifth for measurements in the micro-cultures.

The transferase assay is outlined in Fig. 1. The reagents were as follows:

(1) 6×10^{-4}M uniformly ^{14}C-labeled galactose-1-P, 20 mc/mM (International Chemical and Nuclear Corporation). The commercial ^{14}C-galactose-1-P was purified for use in some experiments in order to remove an alkaline phosphatase resistant contaminant. Descending chromatography was performed on Whatman 3MM paper with butyric acid (100 ml) + 1N ammonium hydroxide (60 ml). The peak was located with a Geiger counter and eluted with a small volume of water.

(2) 2×10^{-4}M UDP-glucose (Sigma Chemical Company).

(3) 1.25×10^{-2}M cysteine, adjusted to *p*H 8.7 with 0.2N NaOH just before use (California Biochemical Corporation).

(4) 0.5M glycine buffer *p*H 8.7 containing 0.01 g Difco gelatin per milliliter.

(5) 6.25×10^{-3}M recrystallized iodoacetamide in 2.5×10^{-3}M NaOH, recrystallization being carried out in 1:1 chloroform:acetone.

(6) Bacterial alkaline phosphatase (BAP) (Worthington Biochemical Corporation) diluted to 30 units/ml in distilled water just before use.

BAP was stored at 4 C. All other reagents were stored frozen.

The microculture monolayers were covered with 0.06 ml of reaction mixture containing: 0.005 ml ^{14}C-galactose-1-P, 0.01 ml UDP-glucose, 0.02 ml cysteine, 0.01 ml glycine buffered gelatin, and 0.015 ml water. Cells from macrocultures were sonicated after suspension in a medium composed of equal volumes of 1.25×10^{-2}M cysteine and glycine-buffered gelatin. Sonicate—0.02 ml was added to 0.04 ml of reaction mixture containing: 0.005 ml ^{14}C-galactose-1-P, 0.01 ml UDP-glucose, 0.01 ml cysteine, and 0.015 ml water. The reaction mixtures were incubated at 10 C. The reaction was stopped by immersion in a boiling-water bath for 1 min. Iodoacetamide (0.1 ml) was added to the cooled reactions; this reagent prevents the inhibition of BAP activity by cysteine. BAP (0.005 ml) was added each hour for 4 hr (0.02 ml total) and incubated at 37 C. The BAP catalyzes the reaction ^{14}C-galactose-1-P $\longrightarrow$ ^{14}C-galactose + P_i. The total reaction mixture was spotted on DEAE paper strips (Whatman DE81), 25 mm by 19 cm, which were prewashed overnight by descending chromatography with deionized distilled water. The entire reaction mixture was applied to the middle of the strip in a single application. The strips were dried and chromatographed descendingly with deionized distilled water for at least 12 hr to elute the ^{14}C-galactose. The strips were dried; the nucleotide-bound ^{14}C, all of which remained within 30 mm on each side of the origin, was determined by counting in a Packard liquid scintillation counter with Liquifluor (Pilot Chemical, Inc.). In this system the efficiency of counting, determined by an internal standard, was about 50%.

Incorporation of galactose was determined by transferring cultures grown in a nonradioactive medium on 25 mm diameter cover slips to media (85 volumes of F_4 lacking both glucose and NCTC 109, 15 volumes fetal bovine serum) containing $^{14}C_1$-galactose (5 mc/mmole, Volk Radiochemical Co.) 0.1 μc/ml, for 4 hr. The cover slips were washed twice in a large volume of 0.9% NaCl and after two successive extractions with 5% TCA (4 C) and ether, the ^{14}C incorporated into the cells was determined by counting the cover slips in a Nuclear Chicago low-background gas flow counter. Additional TCA extractions did not remove any further counts. The total cell protein was determined on the same cover slips after counting.

RESULTS—PART A

Variables of the Transferase Assay. The variables of the enzyme assay were investigated using sonicated preparations of normal fibroblasts.

Enzyme–Time Product Relationship. Decreasing concentrations of normal-cell sonicate were incubated for increasing time so that (relative concentration) × (time) remained constant. This experiment was designed to reveal the presence of dissociable activators and inhibitors within the system and to show that the enzyme being assayed is stable under the given assay conditions. An assay can be considered quantitative only if the amount of product produced remains constant under these conditions, demonstrating that the system obeys this fundamental rule of enzyme action (Neilands *et al.*, 1955). Kinetic experiments performed at a single enzyme concentration and proportionality experiments with a single time of incubation may not reveal enzyme instability or the presence of activators and inhibitors, all of which must be absent

or corrected for in order to quantitatively assay any enzyme. With the method described, in the presence of gelatin at 10 C, the amount of product is constant over a tenfold range in amounts of enzyme actually used in the work reported here (Fig. 2), indicating the quantitative nature of the assay. Under the same conditions, but at 20 C, the amount of product was diminished with increasing times of incubation. This was not due to a low-molecular-weight activator since the decline was observed even after the enzyme preparation had been dialyzed. This suggests that the enzyme is unstable at 20 C.

Further evidence of the temperature instability of the enzyme was obtained by incubating samples of the enzyme at 37, 22, 10, and 0 C for varying intervals and then assaying the residual activity at 10 C. A decline in enzyme activity was detected at 37 and 22 C. Another condition influencing enzyme instability is total protein content.

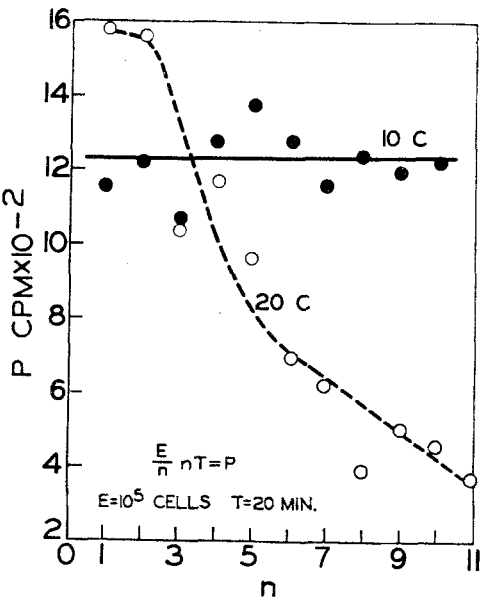

Fig. 2. The relationships among the amount of enzyme, the length of the reaction interval, and the amount of product formed in the transferase determination.

The enzyme is unstable even at 10 C if gelatin is omitted from the reaction mixture. Boiled sonicate can be used in place of gelatin to stabilize the activity.

^{14}C-Galactose-1-P Concentration. The velocity of the transferase reaction was shown to be a function of the ^{14}C-galactose-1-P concentration. In the experiment illustrated in Fig. 3 the concentration of purified ^{14}C-galactose-1-P was varied from 3×10^{-6} to 8×10^{-4}M. Both the substrate concentration and product were experimentally determined since some breakdown of galactose-1-P to galactose and P_i occurred spontaneously. An apparent very high K_m for galactose-1-P of the order of 10^{-2}M is indicated.

UDP-Glucose Concentration. The effect of UDP-glucose concentration on the velocity of the transferase reaction is shown in Fig. 4. The UDP-glucose was varied from 2.4×10^{-7} to 4.3×10^{-3}M. UDP-glucose concentrations above 3×10^{-5}M

inhibited transferase. An apparent K_m of 4×10^{-7}M was calculated for UDP-glucose.

Enzyme Concentration. Figure 5 shows the velocity of the transferase reaction to be directly proportional to cell concentration from 5×10^3 to 10^5 cells.

Kinetics. With the enzyme from 10^5 cells the transferase reaction follows zero-order kinetics for at least 120 min (Fig. 6).

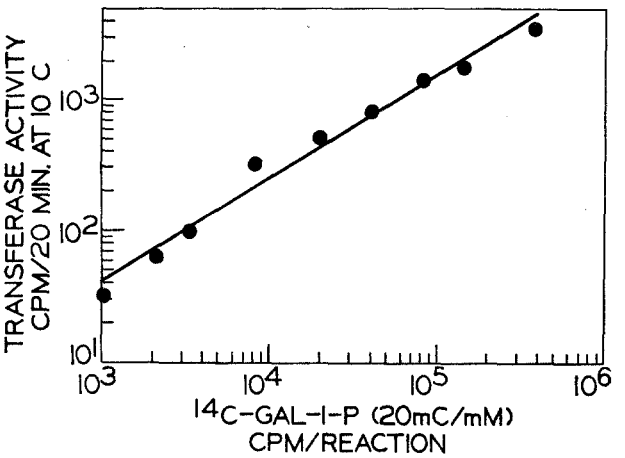

Fig. 3. Transferase activity as a function of the concentration of ^{14}C-galactose-1-P. The reaction mixtures contained normal sonicate equivalent to about 10^5 cells. 1 CPM = 1.7×10^{-4} mμmole.

p*H and Cysteine Requirements.* Cysteine is required for transferase activity when small populations (about 5×10^3 cells) are assayed. With populations near 10^5 cells the addition of cysteine is not required for activity. We have not been able to determine the *p*H optimum for the transferase reaction. The buffers we have tried, glycylglycine, 2-amino-2-methyl-1,3-propandiol, and tris(hydroxymethyl) aminomethane, inhibited the reaction, so that we could not distinguish the effect of hydrogen ion concentration

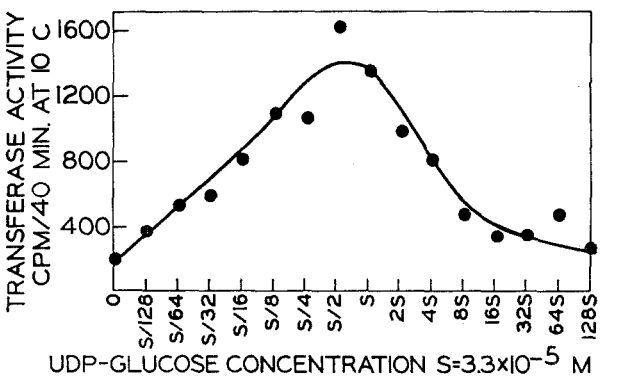

Fig. 4. Transferase activity as a function of the concentration of UDP-glucose. The reaction mixtures contained normal sonicate equivalent to about 10^5 cells.

and inhibitory anion concentration. We used HCl to adjust the *p*H of our tris buffer. Beutler and Baluda (1966) have recently reported that human red cell transferase is inhibited by chloride ions. They found the *p*H optimum to be 8.7—the *p*H at which our assays are performed.

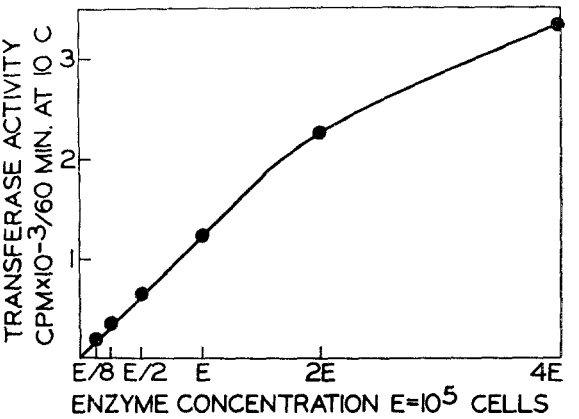

Fig. 5. Transferase activity as a function of enzyme concentration.

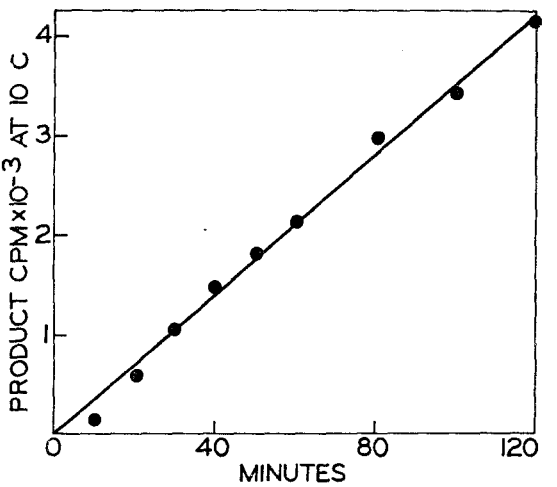

Fig. 6. Kinetics—the amount of product produced as a function of reaction time. The reaction mixtures contained normal sonicate equivalent to about 10^5 cells.

RESULTS—PART B

Observations of Transferase Activity in Cultures of Normal and Mutant Cells

Stationary Phase Cultures. A comparison of the transferase activity in stationary phase cultures of galactosemic, normal, and heterozygous cells is presented in Table I.

These values were determined before we had learned how to stabilize the activity with gelatin at low temperature but we present them because they clearly demonstrate the ability of the assay to distinguish between the three genotypic classes with cultured fibroblasts. They were determined using acetone-fixed monolayers of cells grown in microculture. Enzyme activity was assayed by incubation without gelatin at 37 C for 30 min. Although similar results were obtained in three separate experiments, they might have been partly in error because of the high temperature of incubation, lack of gelatin and/or acetone fixation. Therefore, a comparison was made with one strain of each genotype under the improved conditions (described under methods) using both sonicated preparations and acetone-fixed monolayers. These results, presented

Table I. Specific Transferase Activity in High-Density Cultures of Thirteen Strains of Human Fibroblasts

Genotype	Strain[a]	Transferase activity[b]	Protein[c]	Specific activity[d]	Mean specific activity
+/+	47	5380	50	108	132
Normal	48	7629	57	134	
	60	10012	69	145	
	68	8142	58	141	
+/−	110	2918	54	54	53
Galactosemic heterozygous	108	1501	31	48	
	112	3535	53	67	
	115	2150	43	50	
	116	2773	62	45	
	111	2841	56	51	
−/−	114	301	62	5	4
Galactosemic	113	97	53	2	
	88	204	45	5	

[a] Strains 115 and 116 are heterozygous parents of the galactosemic strain 114, and strains 111 and 112 are heterozygous parents of the galactosemic strain 113.
[b] Transferase activity = CPM product produced at 37 C in 30 min/Microculture.
[c] Protein = Microgram protein culture.
[d] Specific activity = Transferase activity/Protein.

in Table II, confirm the reality of the phenotypic differences between the three genotypes described in Table I and indicate that the two methods of preparing the cells for assay give comparable results. They also confirm the phenotypic differences previously detected in a more indirect manner by Krooth and Weinberg (1961).

Transferase Activity During the Culture Cycle. Studies of glucose-6-phosphate dehydrogenase and β-glucuronidase in our laboratory (DeMars, 1964) have shown that measurement of enzyme activity at a single point in the culture cycle may not always be a reliable way to demonstrate enzymatic differences between strains. Therefore, the transferase activity of the 13 strains listed in Table I was determined during an entire growth cycle. Duplicate determinations of enzyme activity and of total protein for each strain were performed with acetone-fixed microcultures at intervals for eight days after subculture. Since strains of the same genotype gave

similar results, the results for members of the same genotype were averaged in Fig. 7 for clarity of presentation. Early in the growth cycle galactosemic strains showed near normal transferase activity. Unfortunately this experiment was performed before the temperature sensitivity of the enzyme was realized and the results are not free from quantitative error. However, one galactosemic strain was later tested under the better

Table II. A Comparison of Transferase Activity in Sister Cultures Assayed After Sonic Disruption or Acetone Fixation

Strain	Genotype	Acetone-fixed monolayer			Sonicate		
		Transferase activity[a]	Protein[b]	Specific activity[c]	Transferase activity[a]	Protein[b]	Specific activity[c]
60	+/+	1699	45	38	1304	32	41
112	+/−	775	30	26	385	18	21
113	−/−	33	31	1	29	13	2

[a] Transferase activity = CPM product produced at 10 C in 60 min/Reaction mixture.
[b] Protein = Total cell protein (in micrograms)/Reaction mixture.
[c] Specific activity = Transferase activity/Protein.

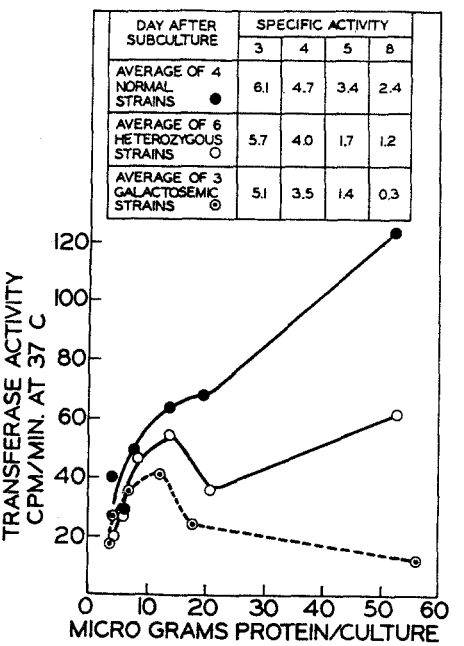

Fig. 7. Transferase activity during the culture cycle. Enzyme activity and total protein were determined on duplicate microcultures on days 1 through 5 and 8 after subculture. The transferase reaction mixtures were incubated at 37 C for 30 min. SPECIFIC ENZYME ACTIVITY = CPM OF PRODUCT/MINUTE OF INCUBATION/MICROGRAM OF PROTEIN.

conditions and similar results were obtained. The activity in this particular strain achieved a maximum activity that was 85% of normal during early growth and eventually fell to less than 8% activity in stationary phase. No transferase activity was found in cell-free microculture vessels incubated and treated in parallel with the test cultures. Also, the culture medium showed no activity before or after incubation with normal cells.

Galactose Utilization by Living Cells. The utilization of galactose by normal, heterozygous, and galactosemic cells was investigated by measuring the rate of incorporation of ^{14}C from $^{14}C_1$-galactose into the TCA insoluble cell fraction during growth. The results in Fig. 8 show no significant difference between normal and heterozygous strains. This was true even when the transferase activity of heterozygous cells had become considerably less than that of control cells in relatively dense populations. Transferase activity may not be the sole factor that limits the kind of incorporation defined here. Homozygous mutant cells incorporated half as much ^{14}C as the

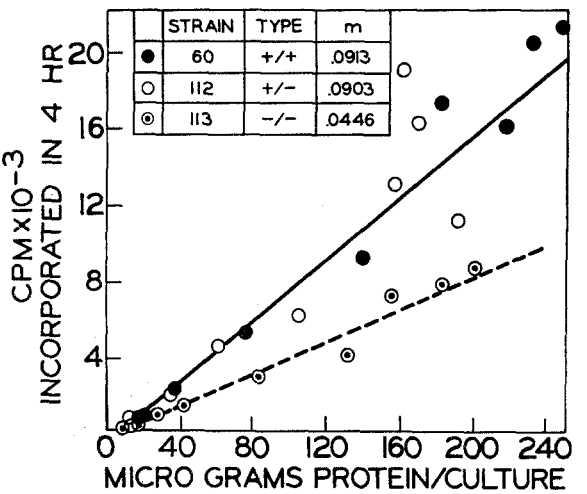

Fig. 8. Incorporation of $^{14}C_1$ from galactose into living cells during the culture cycle. *m* (THE COMPUTED LINEAR REGRESSION) = CPM/MICROGRAM PROTEIN.

Table III. The Effect of Nonradioactive Galactose on the Incorporation of $^{14}C_1$ from $^{14}C_1$-Galactose into Cultured Galactosemic Fibroblasts

Basic medium	Nonradioactive supplements	CPM per cover slip	Percentage of incorporation
$^{14}C_1$-galactose media 0.1 μc/ml −/− cells	Nothing	9309	100%
	5×10^{-3}M Galactose[a]	477	5%
	5×10^{-3}M Glucose	5571	60%

[a] D-Galactose, substantially glucose-free Σ-grade, Sigma Chemical Company.

normal or heterozygous strains. Two other galactosemic strains have also shown extensive incorporation when tested in this manner. To show that this incorporation is indeed due to galactose, and not radioactive contamination, a competition experiment was performed. It is clear from the results in Table III that nonradioactive galactose specifically inhibited the incorporation of $^{14}C_1$-galactose by galactosemic cells.

DISCUSSION

Our objective in developing a new transferase assay was to have a quantitative phenotypic characterization of fibroblasts that was closely relevant to the normal or mutant genes. ^{14}C-galactose-1-P is converted to UDP-^{14}C-galactose in the transferase reaction. Some of this immediate product may be converted to UDP-^{14}C-glucose by the epimerase reaction:

$$\text{UPD-GALACTOSE} \rightleftharpoons \text{UDP-GLUCOSE}$$

The relative abundance of epimerase in fibroblasts compared to red cells has hindered application of the UDP-glucose consumption test (Anderson *et al.*, 1957) to work with cultivated cells. The method described here overcomes this difficulty by trapping both negatively charged radioactive nucleotides resulting from the transferase reaction on DEAE anion exchange paper. The unconsumed substrate of the reaction, ^{14}C-galactose-1-P, is also negatively charged but is effectively eliminated by converting it to ^{14}C-galactose by treatment with alkaline phosphatase prior to chromatographing the reaction mixtures on DEAE paper. Loss of product by recycling UDP-^{14}C-glucose to form ^{14}C-glucose-1-P, which would then be eliminated by alkaline phosphatase treatment, is negligible because the amount of product nucleotide formed is insignificant compared to the amount of nonradioactive UDP-glucose in the reaction mixture.

This phenotypic characterization is direct, in contrast to those used previously. It does not depend on the presence of other enzymes in the cells, as does the determination of CO_2 evolution (Weinberg, 1961; Krooth and Weinberg, 1961), O_2 consumption (Schwarz *et al.*, 1956) ability to grow with galactose as sole hexose (Krooth and Weinberg, 1961) or the ratio of galactose-1-P to UDP-galactose after administration of galactose (Robinson, 1963).

The sensitivity of this method is limited by the specific activity and purity of the ^{14}C-galactose-1-P used. All lots of commercial ^{14}C-galactose-1-P we have used contained from 1 to 2% of an alkaline phosphatase resistant contaminant, which resulted in appreciable background. For this reason ^{14}C-galactose-1-P was used at a concentration which maximized the difference between product formed and background. This concentration was lower than that required for maximum reaction velocity. The amount of product formed during the reaction was small enough so that deviation from zero-order kinetics was not observed. The chromatographic method described for removing this background material was successful with only one lot of substrate. The sensitivity is adequate for 5000 cells, a clone size commonly achieved in our laboratory. Normal fibroblasts have an activity of about 10^{-6} mμmoles UDP-galactose/hr/cell. We can measure 5×10^{-3} mμmoles of UDP-^{14}C-galactose at 20 mc/mmole. Reasonable improvement in the quality of the substrate and prolonged incubation times with sterile reaction mixtures should permit further sensitization.

We are confident that this assay is indeed measuring the activity of transferase since the velocity of the reaction is a function of UDP-glucose and galactose-1-P concentrations and the product formed chromatographs identically with UDP-galactose or UDP-glucose in an ammonium hydroxide-butyric acid system. Also, this assay separated stationary phase cultures into the same three phenotypic classes as did the erythrocyte UDP-glucose consumption test used in diagnosing the donors.

The appearance of near normal levels of transferase activity in mutant strains during rapid growth is most likely a real characteristic of many galactosemic mutations, since it was observed in three apparently unrelated but "typical" galactosemics and six unrelated galactosemic heterozygotes. We are presently investigating the nature of this mutation to determine whether it affects the structural gene for transferase or a regulatory mechanism. The loss of activity in crowded populations could be due to instability of the mutant enzyme under certain varying physical conditions of the cultures. Two mutants of *Neurospora crassa* with reduced tryptophane synthetase activity exemplify other effects of structural alterations which could be responsible for the variation in the specific activity of the transferase produced by galactosemic cells. The tryptophane synthetase produced by one of these mutants is more sensitive to heavy metal inhibition than the normal enzyme (Suskind and Kurek, 1957). The other mutant produces an enzyme which requires two cofactors not required by the wild-type enzyme for the reaction (DeMoss and Bonner, 1959).

Another mutation studied in cultured human cells also affects the specific activity of an enzyme as a function of population density. Krooth's studies of orotidine-5'-monophosphate decarboxylase indicate that the specific activity of this enzyme increases with population density. The data suggested that the rate of this increase might be greater in cells cultured from patients with orotic aciduria (Krooth, 1964). Dr. Krooth suggests that this mutation may be at a regulator locus. In our case the specific transferase activities of all three genotypes decline as a function of time after subculture (see table of specific activities in Fig. 7) but they do so at different rates, so that the differences between normal and mutant strains increase. Examining the electrophoretic behavior of the transferase produced by normal and galactosemic strains during rapid growth with the starch gel electrophoresis system developed by Mathai and Beutler (1966) may reveal a structural difference if one exists.

Galactosemic strains utilize galactose, as determined by the incorporation experiment, at half the normal rate throughout the culture cycle. This suggests that either transferase is active within the living cell during stationary phase or it is not the primary pathway for galactose incorporation in galactosemia. Two other pathways for galactose metabolism are known, one mediated by uridine-diphosphate galactose pyrophosphorylase (Isselbacher, 1958), the other by galactose dehydrogenase (Cuatrecasas and Segal, 1966). We do not know if these pathways are operative in human fibroblasts. Further discussion of these data would be futile here. Fractionation of the cells in order to determine the metabolic fate of the galactose is the next step necessary to arrive at a precise definition of "incorporation" in these experiments.

We would like to select for or against genetically altered cells in this system. The surprisingly high incorporation of galactose by the mutant strains might be expected to hinder direct selection for normal cells by relying on their ability to grow in media containing galactose as the only hexose. However, the results of others (Krooth and Weinberg, 1961) indicate that the ability of homozygous mutants to incorporate galactose, in the broadly defined sense used here, does not permit them to satisfy their hexose requirement for growth in galactose alone. Direct selection is still a reasonable possibility but continues to be hampered by technical difficulties (Krooth, 1964, 1965, and 1966). The incorporation of ^{14}C-galactose by mutant cells also means that the

detection of "Gal$^+$" cells in "Gal$^-$" populations by simple application of the autoradiographic technique to cells after they have been grown in nonselective medium containing ^{14}C-galactose may give less than black vs. white phenotypic differences. An alternative to these selective methods, direct visual selection in populations of living cells, has been discussed elsewhere (see discussion in DeMars and Leroy, 1966). The work reported here should become useful once a workable selective procedure is devised. Meanwhile, it can be used now to investigate the effects of galactosemia mutations on the transferase enzyme.

ACKNOWLEDGMENT

We are grateful to our colleagues G. H. Echols, Leonard Isaacs, Nancy Couse, and Oliver Smithies for valuable suggestions and gifts of material used in our work.

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