though normal growth of this organism could have been initiated with a very much smaller inoculum.

The cultures were clarified by centrifugation, acidified by adding 2 ml of concentrated sulfuric acid to each, and steam-distilled to remove volatile acid. Titratable acid, amounting to 165 and 230 μequivalent/ml of culture, was produced by the dependent and independent strains, respectively. Acidity lost during steam distillation amounted to 140 and 138 μequivalent/ml, respectively, for the two cultures (*4*). Ammonium sulfate and sulfuric acid were added to the residual cultures to make each final solution 1*N* with respect to sulfuric acid and 30 percent with respect to ammonium sulfate. Each solution was then extracted with seven consecutive 15-ml portions of isopropyl ether-butanol mixture (2/1 by volume), and titratable acidity and growth-promoting activity were determined in a 1-ml aliquot of each extract.

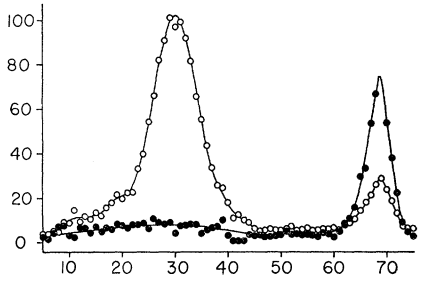

Fig. 1. Distribution of titratable acid (open circles) and growth-promoting activity (solid circles) in extracts of a *Lactobacillus casei* 7469 culture after 70 transfers in the following solvent system: upper phase isopropyl ether-butanol 2/1 by volume; lower phase 30 percent ammonium sulfate in 1*N* sulfuric acid. The values on the vertical scale represent either microequivalents of titratable acid (open circles) or units of growth-promoting activity (defined in Table 1) (solid circles) in the upper-phase solution (10 ml) of each cell. The numbers on the horizontal scale designate the consecutive cells of the counter-current extraction apparatus. The seven extracts of the *L. casei* 7469 culture (Table 1) were combined, evaporated nearly to dryness under reduced pressure, dissolved in 50 ml of upper-phase solvent, and distributed, 10 ml per cell, into cells 4, 5, and 6 at the outset of the counter-current extraction.

Table 1. Titratable acid and growth-promoting activity in extracts of cultures of *Lactobacillus casei*.

Extract No.	Strain 280–16A		Strain 7469	
	Titratable acid* (meq)	Growth-promoting activity (units)†	Titratable acid (meq)	Growth-promoting activity (units)
1‡	0.64	< 4	0.85	169
2	1.09	< 7	1.32	244
3	1.05	< 7	1.24	124
4	0.93	< 7	1.19	87
5	0.90	< 7	1.17	60
6	0.88	< 7	1.15	46
7	0.86	< 7	1.12	38

* Calculated from titrations with 0.01*N* sodium hydroxide. Phenolphthalein indicator, used with extracts 1 through 3 of the 280–16A culture, was replaced with brom thymol blue indicator for the remaining extracts of both cultures since the latter gave a sharper end point (but somewhat low titration values).

† One unit of growth-promoting activity is an amount equivalent to the growth-promoting activity 1 μmole of D-lactic acid (used as standard in the microbiological assays).

‡ During the first extraction, part of the nonaqueous solvents dissolved in the aqueous phase, and for this reason the first extract was smaller in volume and otherwise is not directly comparable to the subsequent extracts.

The results, shown in Table 1, make it fairly evident that growth-promoting activity is not associated with the major fraction of titratable acid, which appears, as would be expected, to have the correct distribution coefficient for lactic acid (0.6 in this solvent system, according to the results of a preliminary experiment with the purified compound) in either culture. That essentially none of the growth-promoting activity produced by the independent strain is associated with lactic acid is evident from the data obtained by counter-current extraction of three-fifths of the remaining combined extracts from the culture medium of this organism (Fig. 1). The solvent system employed for this extraction (carried through 70 transfers) was the same as that for the preliminary extraction. It may be seen (Fig. 1) that the distribution of the major titratable component distribution coefficient, 0.56) is close to that which could have been predicted from the distribution coefficient of lactic acid. A much smaller component (distribution coefficient, 10.6) is seen to be associated with the growth-promoting activity (Fig. 1). That this growth-promoting material was not formed artifactitiously is evident from its absence in the extracts of the dependent culture, which was treated as a control (Table 1). The growth-promoting potency of the active component appears to be about three times that of D-lactic acid when growth-promoting units (defined in Table 1) are compared with titration equivalents (Fig. 1). The high activity of the material, together with its relatively high distribution coefficient and other solubility properties, suggest that it is either a higher (by several carbons in chain length) homolog of D-lactic acid or a closely related analogous acid. Isolation of larger amounts of this material for exact identification is in process.

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3. This investigation (No. 120) was aided by grants from the National Multiple Sclerosis Society, the U.S. Public Health Service, and the University of California. We are indebted to Evelyn Brown and Audree Fowler for technical assistance.
4. The calculated acetic acid content of the acidified cultures was 146 μequivalent/ml derived from sodium acetate included in the medium. It appears, therefore, that 94 to 96 percent of the available acetic acid was removed by the steam distillation. Probably only negligible amounts of lactic acid and growth-promoting acid, if any, were removed by this process.

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Induction of Enzymes of the Galactose Pathway in Mutants of Saccharomyces cerevisiae

Induced biosynthesis of enzymes is dependent on the presence of a stereospecific inducer, which is not necessarily a substrate (*1*). In *Pseudomonas* it was shown that a sequential adaptation of a series of enzymes of a metabolic pathway takes place in the presence of the first (or often an intermediary) substrate of a chain of inducible enzymes (*2*). In the pathway

$$A \xrightarrow{\text{I}} B \xrightarrow{\text{II}} C \xrightarrow{\text{III}} D$$

enzyme II will arise in response to the formation of product *B*, and enzyme III will be formed when product *C* accumulates, and so forth.

In the present study (*3*, *4*) the inducible properties of the enzymes of galactose metabolism are considered in relation to the problem of sequential induction. The formation of these enzymes in response to galactose cannot be explained in terms of this pattern.

The galactose pathway (the old "galactozymase complex") which effects the complete transformation of galactose to glucose has now been completely established (*5–8*) and can be summarized as follows (*4*):

$$\text{Galactose} + \text{ATP} \xrightarrow{\text{galactokinase}} \text{Gal-1-P} + \text{ADP} \quad (1)$$

$$\text{Gal-1-P} + \text{UDPG} \underset{\text{Gal-1-P uridyltransferase}}{\rightleftharpoons} \text{UDPGal} + \text{G-1-P} \quad (2)$$

$$\text{UDPGAL} \underset{\text{(DPN)}}{\overset{\text{UDPGal-4-epimerase}}{\rightleftharpoons}} \text{UDPG} \quad (3)$$

Sum: $\text{Galactose} + \text{ATP} \rightleftarrows \text{G-1-P} + \text{ADP}$

The initial formation of the catalytical amount of uridine diphosphoglucose necessary to start reaction 2 is provided by a fourth reaction:

$$\text{UTP} + \text{G-1-P} \underset{\text{UDPG pyrophosphorylase}}{\rightleftharpoons} \text{UDPG} + \text{PP} \quad (4)$$

In the present study two haploid mu-

tants of *Saccharomyces cerevisiae* which are not able to ferment galactose were used (*9*). One of the mutants was considered to be galactokinaseless (genotype g_1G_2 (*10*), while the other one is apparently defective in a system involved in the transport of galactose (genotype G_1g_2) (*11*).

The activities of the enzymes involved in the galactose pathway were determined by means of recently developed methods (*8, 12, 13*).

Galactokinase activity was found to be present in G_1g_2 cells when it was induced by galactose, but it was always completely absent in g_1G_2 cells (see Table 1). These results are in complete agreement with the finding that, in yeast, the galactokinase activity is under the control of the gene G_1. The role of the G_2 gene in the "active transport" of galactose is under investigation.

By the addition of galactose either to the cells growing on glucose-salts medium or to cells suspended in buffered acetate solution, a very striking synthesis of the galactose enzymes takes place even in the galactokinaseless mutant (see Table 1). Although α-galactose-1-phosphate, the normal substrate for α-galactose-1-phosphate uridyl transferase, is not formed, or at least not in detectable amounts, this enzyme and the subsequent enzyme, uridine diphosphogalactose-4-epimerase, are nevertheless induced. The absence of uridine diphosphogalactose-4-epimerase in unadapted yeast was unexpected, since uridine diphosphoglucose, the other substrate for this enzyme, is always present (*6*). The fact that the α-galactose-1-phosphate uridyl transferase was induced without the formation of any detectable amount of its substrates was also contrary to expectation; with techniques developed in this laboratory (*12*), less than 0.0002 μmole of α-galactose-1-phosphate or uridine diphosphogalactose formed per milligram of protein per hour could have been detected.

It is, therefore, difficult to explain the observed phenomena on the basis of sequential adaptation unless one assumes that a few molecules of α-galactose-1-phosphate are able to induce α-galactose-1-phosphate uridyl transferase. Furthermore, an even smaller number of uridine diphosphogalactose molecules would, in turn, induce the uridine diphosphogalactose-4-epimerase. A more reasonable hypothesis is that free galactose itself acts as the "gratuitous," and multi-, inducer of the biosynthesis of two successive enzymes of the galactose pathway in the galactokinaseless mutant. A more detailed paper describing additional data which support this hypothesis is in preparation.

It should be emphasized that in the present case galactose appears to initiate the induction of at least three enzymes, catalyzing entirely different types of reactions: galactokinase, catalyzing phosphorylation of the reducing group of galactose; α-galactose-1-phosphate uridyl transferase, catalyzing the transfer of a uridyl group; and uridine diphosphogalactose-4-epimerase, catalyzing an inversion. Free galactose has already been shown to be an inducer for β-galactosidase (*14*) and β-galactoside permease (*15*) in *Escherichia coli*.

A number of other pentoses and hexoses have been tried, but thus far only galactose has been found to serve as an inducer.

Note added in proof: After the present paper was submitted, Mills *et al.* reported that uridine diphosphogalactose-4-epimerase is present in unadapted *Saccharomyces fragilis* [*Biochim. et Biophys. Acta*, **25**, 521 (1957)]. We repeated the same type of experiments reported here, but used *S. fragilis*, and the results, in complete agreement with the present findings with *S. cerevisiae*, show that uridine diphosphogalactose-4-epimerase cannot be detected in unadapted yeasts.

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3. I am grateful to H. M. Kalckar for his helpful suggestions and constant interest during the course of this work.
4. The following abbreviations are used: ADP and ATP, adenosine di-, and tri-, phosphate, respectively; Gal-1-P, α-galactose-1-phosphate; G-1-P, α-glucose-1-phosphate; UDPG; uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; PP, inorganic pyrophosphate; UTP, uridine triphosphate.
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16. The culture medium contained, per liter: $MgSO_4$, 1.4 g; KH_2PO_4, 2.0 g; NaCl, 1.0 g; $(NH_4)_2SO_4$, 7.0 g; $CaCl_2$, 0.8 g; $FeCl_3 \cdot 6H_2O$, 0.4 g; *l*-tryptophan, 10.0 mg; *l*-methionine, 10.0 mg; *l*-histidine HCl, 10.0 g; glucose, 5.0 g. Galactose was added, when indicated, from the beginning of the growth.
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Table 1. Specific activities of "galactose enzymes" in an uninduced and induced galactokinaseless strain of *Saccharomyces cerevisiae*. Cells were grown aerobically for 48 hours at 25°C in glucose synthetic medium(*16*) (with and without galactose); activities were determined on a dialyzed "Nossal" (*17*) extract; substrates for steps 1, 2, 3, and 4 were galactose, Gal-1-P, UDPGal, and UDPG, respectively.

Galactose in growth medium (mg/ml)	Specific activity [(μmole × 10^{-4})/(min × mg)]			
	Galactokinase (step 1)	Gal-1-P uridyl transferase (step 2)	UDPGal-4-epimerase (step 3)	UDPG pyrophosphorylase (step 4)
0	0	0.1	0	91.7
1	0	89.6	57.0	125.0

Apparent Shifts of Absorption Bands of Cell Suspensions and Selective Light Scattering

Absorption spectra of cell suspensions are usually determined by the measurement of transmitted light beams. Cell suspensions attenuate an incident beam in two ways—by absorption and by scattering. The effect of scattering is almost always of the same order of magnitude as that of absorption. It is generally assumed that scattering varies gradually and uniformly with wavelength and that it therefore does not greatly alter the positions and shapes of absorption bands. Recently, however, it was found that pigmented algal cells scatter light with a strong spectral selectivity (*1*). Sharp scattering peaks occur on the long wavelength sides of the major absorption bands. The experiments described in this report were carried out to determine whether light scattering can seriously distort the absorption spectra of cells (*2*).

Absorption spectra of a suspension of the green alga *Chlorella pyrenoidosa* were measured in two different ways, one designed to maximize, the other to minimize, the influence of scattering. Both measurements were made with a Beckman DK-2 recording spectrophotometer.

Three possible arrangements of the optical system are shown in Fig. 1. Arrangement *a* is the usual one. The photocell is so far from the sample that it collects only light transmitted directly and that scattered within about ± 3 de-