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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References and Notes

1. J. Monod and M. Cohn, *Advances in Enzymol.* 13, 67 (1952).
2. R. Y. Stanier, *Bacteriol. Rev.* 14, 179 (1950); M. Suda, O. Hayaishi, W. Oda, *J. Japan Biochem. Soc.* 37, 351 (1950).
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.
5. H. W. Kosterlitz, *J. Physiol.* (*London*) 33, 34 (1938).
6. L. F. Leloir, *Arch. Biochem.* 33, 186 (1951).
7. H. M. Kalckar, B. Braganca, A. Munch-Petersen, *Nature* 172, 1039 (1953); A. Munch-Petersen *et al.*, *ibid.* 172, 1036 (1953).
8. A. Munch-Petersen, H. M. Kalckar, E. Smith, *Kgl. Danske Videnskab. Selskab Biol. Medd.* 7, 3 (1955).
9. I am indebted to D. C. Hawthorne for a generous supply of these two mutants.
10. N. Nelson, thesis, University of Washington 1955.
11. H. C. Douglas and F. Condie, *J. Bacteriol.* 68, 662 (1954).
12. E. S. Maxwell, H. M. Kalckar, R. M. Burton, *Biochim. et Biophys. Acta* 18, 464 (1955).
13. H. M. Kalckar, E. Anderson, K. J. Isselbacher, *ibid.* 20, 262 (1956).
14. J. Monod, G. Cohen-Bazire, M. Cohn, *ibid.* 7, 585 (1951).
15. A. B. Pardee, *J. Bacteriol.* 73, 376 (1956).
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.
17. P. M. Nossal, *Australian J. Exptl. Biol.* 31, 583 (1953).

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17 September 1957

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-

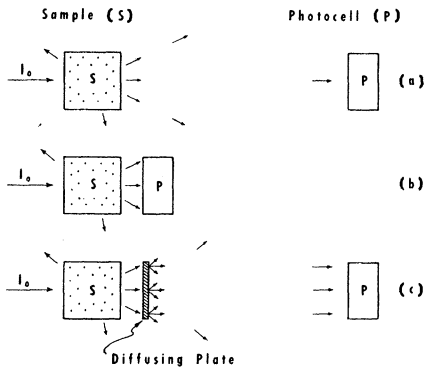

Fig. 1. Schematic diagrams of part of optical system of spectrophotometer.

grees. This arrangement gives an absorption spectrum which is the one most severely influenced by scattering. Arrangement *b* permits the photocell to collect light scattered angles up to about 30 to 50 degrees. Arrangement *c*, suggested by Shibata *et al.* (*3*), is equivalent to *b* with respect to the collection of scattered light and is experimentally more convenient. A diffusing plate, inserted behind the sample, causes a small but representative part of the light striking it to enter the photocell. Arrangement *c*, with identical diffusing plates made of opal glass placed behind the sample and the blank vessel, was used to minimize the influence of scattering.

The only spectrophotometer settings which were different for the two arrangements were those for the amplification of the photocell response and the band halfwidth (5 mμ for *a* and 7 mμ for *c* at a wavelength of 690 mμ; less at shorter wavelengths). Controls indicated that these differences did not influence the results. As a further check of the instrument and method, absorption spectra of chlorophyll in acetone were measured

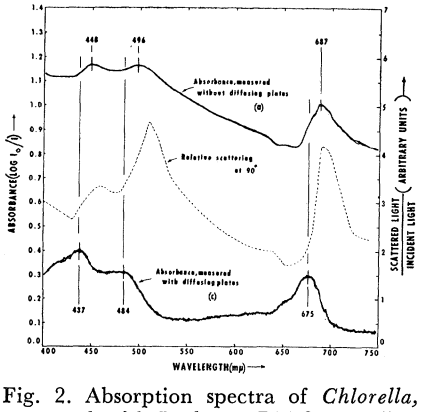

Fig. 2. Absorption spectra of *Chlorella*, measured with Beckman DK-2 recording spectrophotometer; arrangements *a* and *c* (shown in Fig. 1) were used. Both absorption curves are reproductions of original spectrophotometer recordings, each of which was made in triplicate. Center curve: scattering by *Chlorella* at 90 degrees. [From P. Latimer and E. Rabinowitch (*1*)]

with arrangements *a* and *c*. The shapes and positions of the bands on the two curves were identical, but the absorption between the bands differed slightly, probably because of multiple reflections between the diffusing plate and the sample vessel.

Figure 2 shows the absorption curves of a *Chlorella* suspension (10^{-3} ml of cells per milliliter) obtained with arrangements *a* and *c* and also the previously reported scattering curve of this organism. The latter curve is for light scattered at 90 degrees but is qualitatively representative of light scattered at most angles.

Of particular interest are the differences of more than 10 mμ between the positions of the major bands on the two absorption curves. Similar differences are found in the absorption curves published by Shibata *et al.* (*3*); however, these authors emphasized only the sharpness of the absorption bands as measured with diffusing plates and did not draw attention to the differences in the positions of the peaks.

Curve *c* is in reasonably good agreement with other absorption spectra reported for *Chlorella* (*1, 3, 4*); presumably the positions and shapes of the bands on it are approximately correct. The bands on curve *a*, however, seem to represent the *sum* of the scattering bands and the absorption bands (on curve *c*) of *Chlorella* which occur at different wavelengths. Light interference effects, such as those observed by Lothian and Lewis (*5*) in similar studies of red blood cells, may also have contributed to the differences between our "absorption" curves. Neither mechanism by itself can explain all related experimental results that have been reported to date. However, the asymmetry of the distorted absorption bands on curve *a* about the actual absorption maxima on *c* indicates that anomalous dispersion, which is also asymmetrical about absorption bands and which causes selective scattering, is of primary importance.

We are investigating the effects of scattering with a special spectrophotometer that allows the making of measurements of light transmitted or scattered at different angles. Some of the results, which confirm those shown in Fig. 2, should be useful in interpreting this distortion phenomenon.

Chlorella cells of the same strain but from other cultures gave quantitatively different results. While the red absorption maximum of all cells occurred at 675 mμ on the *c* curves, the position of this maximum on the *a* curves varied, with the culture, from 683 mμ to 691 mμ. The bands at the longest wavelengths were obtained with the cells which appeared to be in the rapid growth phase.

We also measured pairs of absorption curves for suspensions of chloroplasts and very small chloroplast fragments (*6*). The chloroplasts (average dimension 5 to 6 μ) gave a curve like some of those of *Chlorella*. The suspension of small fragments, however, behaved more like chlorophyll in solution; its bands on curves *a* and *c* were identical in shape and position.

The optical effect described here can significantly distort the positions and shapes of absorption bands of cells and their components. Some published absorption spectra of cells may show the influence of this effect. The influence would vary with the optical system of the spectrophotometer and with the scattering characteristics of the particular suspension.

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References and Notes

1. P. Latimer and E. Rabinowitch, *J. Chem. Phys.* 24, 480 (1956); also P. Latimer, thesis, University of Illinois, 1956.
2. I thank C. S. French, J. H. C. Smith, and H. W. Milner for their helpful suggestions.
3. K. Shibata, A. A. Benson, M. Calvin, *Biochim. et Biophys. Acta* 15, 461 (1954).
4. R. Emerson and C. M. Lewis, *Am. J. Botany* 30, 165 (1943).
5. G. F. Lothian and P. C. Lewis, *Nature* 178, 1342 (1956).
6. Spinach chloroplasts were prepared with a Waring blender and washed. The small fragments were obtained by forcing chloroplasts through the needle valve used in the method of Milner *et al.* (*7*). The large fragments were then removed by centrifugation, which left a clear green "suspension" of small fragments.
7. H. W. Milner, N. S. Lawrence, C. S. French, *Science* 111, 633 (1950).

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6 November 1957

Effect of Reserpine and Promazine on Diphosphopyridine Nucleotide Synthesis in Liver

It has been found by Kaplan *et al.* that the injection of nicotinamide into mice results in large increases of the levels of diphosphopyridine nucleotide (DPN) in tissue (*1*). The administration of "tranquilizing" agents, such as reserpine (*2*) and promazine (*3*), prior to the injection of nicotinamide results in the level of DPN in the liver being maintained at an elevated concentration for a prolonged period of time. This report (*4*) presents details of this observation and discusses some of their implications.

Hybrid male mice [(BALB/c An × DBA/2J)F_1, 8 to 10 weeks old, of 20 to 25 g body weight] were divided randomly into groups. Reserpine was administered subcutaneously 4 hours prior to the intraperitoneal injection of nicotinamide. Animals were sacrificed by cervical fracture at different time in-