rate constant k_{-4}. Modifiers that increase the rate of the isomerization process E'S⇌E''S will cause a rate increase at low substrate concentrations and co-operativity with respect to the substrate would be abolished if the value of k_{+2} is increased sufficiently for the inequality $k_{+2} \gg k_{+3}$ to hold.

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Regulation of the Concentration or Activity of Pyruvate Kinase in Yeasts and its Relationship to Gluconeogenesis

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Marked decreases in the concentration of pyruvate kinase have been observed in certain yeasts grown in conditions of gluconeogenesis (Ruiz-Amil, de Torróntegui, Palacián, Catalina & Losada, 1965; Gancedo, 1966). A similar behaviour with low glucose concentration in the medium has also been claimed (Hommes, 1966). Recently Hess, Haeckel & Brand (1966) found a strong activation by FDP* of the pyruvate kinase of brewer's yeast. We now report that two alternative mechanisms for the regulation of pyruvate kinase occur in different yeasts, one controlling its concentration and the other its activity. Either of these regulatory mechanisms permits a 'shutting off' of pyruvate kinase at the level of the phosphoenolpyruvate crossroad in gluconeogenesis.

Saccharomyces cerevisiae and *Candida utilis* are compared in Table 1. The former does not exhibit changes in the concentration of pyruvate kinase in relation with the carbon source, and the pyruvate kinase in the extracts is utterly insufficient to account for the glycolytic capacity of this yeast. Its activation by FDP qualitatively confirms that found by Hess *et al.* (1966) with brewer's yeast. Quantitatively it is about five times as great; this could be due to difference in the organism or in the test conditions. On the other hand, *C. utilis* has a large amount of pyruvate kinase when grown on glucose, but this amount is markedly smaller when grown on ethanol, and in no case was the activity increased by addition of FDP. Intermediate values of pyruvate kinase have been observed in *C. utilis* grown on glycerol. In *Rhodotorula glutinis* the pyruvate-kinase concentration also changes markedly with the carbon source (Ruiz-Amil *et al.* 1965), and again we have found that the enzyme activity is not affected by FDP.

The low concentration of pyruvate kinase in ethanol-grown *C. utilis* is accompanied by virtual inability to ferment glucose when transferred to a glucose medium (Table 1). The fact that the gradual regaining of the ability to ferment glucose was completely prevented by Actidione suggests that protein synthesis is involved (Siegel & Sisler, 1964), although more work would be required to pinpoint the limiting step.

Reciprocal changes in the concentrations of phosphofructokinase and fructose 1,6-diphosphatase have been observed in yeast and seem to be a factor in the regulation of the shift from glycolysis to gluconeogenesis, or vice versa, at the level of this pair of antagonistic irreversible enzymes (Gancedo, Salas, Giner & Sols, 1965). Gluconeogenesis from oxaloacetate precursors involves in yeast phosphoenolpyruvate carboxykinase (Ruiz-Amil *et al.* 1965). In these conditions phosphoenolpyruvate is a major crossroad, and an active pyruvate kinase would be a considerable hindrance. It appears that in yeasts the required 'shutting off' of pyruvate kinase can be accomplished either by a marked decrease in the concentration of enzyme or through a marked dependence for its activity on a concentration of FDP that could be reached in glycolysis but

* Abbreviation: FDP, fructose 1,6-diphosphate.

Table 1. *Pyruvate kinase and glucose fermentation in yeasts grown on different carbon sources*

Expt. A. Growth medium and preparation of extracts were as described by Gancedo *et al.* (1965). Pyruvate kinase was assayed spectrophotometrically as described by Bücher & Pfleiderer (1955), but with 40mM-tris–HCl buffer, pH 7·6, at room temperature (approx. 22°). Results were corrected for blanks without phosphoenolpyruvate and without or with FDP, as appropriate. The numbers of experiments are given in parentheses. Expt. B. Anaerobic fermentation was followed in Warburg vessels containing initially 10mg. of yeast in 3ml. of growth medium with glucose (30mM), at 30°. The reported rates were taken as the average value from 20min. intervals (10min. before to 10min. after the times indicated), and are referred to the initial amount of yeast. Vessels containing 9μg. of Actidione were run in parallel.

			Pyruvate-kinase activity (μmoles/g. fresh wt. of yeast/min.)		
	Yeast	Carbon source	Without FDP	+FDP (1mM)	Activation factor
Expt. A	*S. cerevisiae*	Glucose	~0·2 (2)	40 (2)	~170
		Ethanol	~0·2 (2)	30 (2)	
	C. utilis	Glucose	40 (11)		Nil (<1·2)
		Ethanol	7 (9)		

				Glucose fermentation (μmoles of CO_2/g. fresh wt. of yeast/min.)				
	Yeast	Carbon source	Actidione	30min.	2hr.	3hr.	4hr.	5hr.
Expt. B	*C. utilis*	Glucose	—	75	—	—	—	—
			+	28	—	—	—	—
	C. utilis	Ethanol	—	1·7	3·0	9·0	18	28
			+	1·0	0·7	1·0	1·0	0·9

not in gluconeogenesis. We have found FDP concentrations of approx. 1·5 and 0·03μmoles/g. fresh wt. in *S. cerevisiae* growing on glucose and ethanol respectively as carbon source. The decrease in pyruvate kinase in liver in starvation found by Krebs & Eggleston (1965) was also interpreted in relation to the gluconeogenic activity of the liver in this condition. In rat skeletal muscle, where there is no by-pass for the reversal of the pyruvate-kinase reaction (Salas, Viñuela, Salas & Sols, 1964), there is neither a decrease in the concentration of pyruvate kinase in starvation (Tanaka, Harano, Morimura & Mori, 1965), nor is the enzyme sensitive to FDP (Hess *et al.* 1966). Moreover, Tanaka *et al.* (1965) have identified two isoenzymes of pyruvate kinase in rat tissues, one of which is peculiar to the liver and varies with the nutritional state of the animal. The apparent dependence of the pyruvate-kinase concentration in a *Candida* strain on the concentration of glucose in the medium, reported by Hommes (1966), probably involved depletion of glucose and utilization of early accumulated ethanol (Polakis & Bartley, 1965). Indeed, we have found high concentrations of pyruvate kinase in *C. utilis* grown in a medium with an initial glucose concentration of 0·5% and harvested when the residual glucose was as low as about 0·1%.

These considerations support the conclusion that the control of pyruvate kinase is related to the potential switch-over to gluconeogenesis rather than intrinsic to glycolysis.

FDP specifically affects a variety of bacterial enzymes, apparently as an allosteric regulator [activation of the lactate dehydrogenase of *Streptococcus bovis* (Wolin, 1964), of the ADP-glucose pyrophosphorylase of *Escherichia coli* (Preiss, Shen, Greenberg & Gentner, 1966) and of the phosphoenolpyruvate carboxylase of *Salmonella* (Sanwal & Maeba, 1966), inhibition of glycerol kinase of *E. coli* (Zwaig & Lin, 1966), and a labilization of β-galactosidase of *E. coli*, possibly involved in its catabolite repression (Gert & Mandelstam, 1966)]. The activation of yeast pyruvate kinase is the first known implication of FDP in the regulation of yeast metabolism, where several regulatory effects involving glucose 6-phosphate have been described [activation of glycogen synthetase (Algranati & Cabib, 1962), inhibition of hexose transport (Sols, DelaFuente, Viñuela & Heredia, 1963), repression of alcohol dehydrogenase, malate dehydrogenase, isocitrate lyase and malate synthase (Witt, Kronau & Holzer, 1966) and of fructose 1,6-diphosphatase (C. Gancedo, J. M. Gancedo & A. Sols, unpublished work)]. Evidence of nutritional induction of pyruvate kinase by glucose in *Rhodotorula glutinis* has been obtained by M. J. Fernández, L. Medrano, M. Ruiz-Amil and M. Losada (unpublished work). The actual inducer is likely to be a metabolite below fructose 6-phosphate along the glycolytic pathway,

since either glucose or glycerol as carbon source give in *C. utilis* high concentrations of pyruvate kinase. Then the inducer could well be FDP, the metabolite that activates pyruvate kinase in other yeasts.

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Biosynthesis of Thyroglobulin and Sub-units in the Rat Thyroid Gland *in vivo*

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Seed & Goldberg (1963, 1965*a*), in studies of protein biosynthesis in thyroid tissue slices, have shown that labelled amino acids are incorporated into proteins sedimenting more slowly than thyroglobulin during the initial minutes of incubation, before any label could be detected in thyroglobulin itself. Because of the limitations of experiments with thyroid slices *in vitro*, specifically in view of the possibility of artifacts (Seed & Goldberg, 1965*b*; Nunez, Mauchamp, Macchia & Roche, 1965), we chose to study the biosynthesis of thyroid proteins in intact animals. The present communication describes the sequence of labelling of soluble proteins of the thyroid glands of rats injected with labelled amino acid.

Methods. Male Sprague–Dawley rats (weighing 150–200g.) were fed on a low-iodine diet (General Biochemicals, Chagrin Falls, Ohio, U.S.A.) for 10–14 days. L-[U-^{14}C]Leucine (specific activity greater than 200mc/m-mole) was injected intraperitoneally in a dose of 15μc/animal. At intervals between 30min. and 48hr. after injection groups of two or three rats were killed by exsanguination under ether anaesthesia.

The thyroid glands were chilled to 4° within 1min. after removal and homogenized in an all-glass homogenizer in cold phosphate-buffered saline (0·15M-NaCl–0·01M-potassium phosphate buffer, pH 6·8). Care was taken to avoid freezing the glands and homogenates.

Previous work in this Laboratory has shown that the concentration of diffusible ^{14}C-labelled material in the blood of rats reaches a maximum within 10min. and falls to less than 15% of the maximum within 60min. after a single intraperitoneal injection of [^{14}C]leucine. Therefore the present studies may be considered pulse-labelling experiments.

Results and discussion. The sequence of labelling of soluble proteins extracted from the thyroid glands is illustrated in Fig. 1. The sedimentation pattern of extracts dialysed at pH 6·8 (top panels) showed a progressive shift in the major radioactive peak from 12s at 1hr. to 16s at 4hr. and 18s at 20hr. The relative specific radioactivity, defined as the ^{14}C/extinction ratio, of the 12s protein exceeded that of all other soluble proteins both at 30min. (not shown in Fig. 1) and at 1hr. after the injection