C^{14}-RNA is mostly incorporated into the cytoplasm after 1 h following the injection and, to a very small extent, into the nucleus and the chromosomal regions. After 6 h, the activity has increased in cytoplasm and also the nucleolus. Many observations suggest that the ingested foreign DNA becomes part of the nuclear chromatin[9,10].

In our experiments, the labelling, after H^3-DNA injection, is found in the cytoplasm; later on, it accumulates in the nucleus, on the polytene chromosomes. KONG and FICQ[11] observed that after injection of phage C^{14}-DNA into newts, radioactivity can be found in the lampbrush chromosomes of the oocytes. It is reported that when H^3-DNA (from *E. coli*) is introduced into the blastomeres of *Pleurodeles* embryos, radioactivity is soon found in the nuclei and can later be distributed between the daughter nuclei[12].

The incorporation of macrophage RNA into lymph nodes has been studied by FISHMAN et al.[13]. LACOUR et al.[14] have observed that Ehrlich ascites tumor cells absorb large amounts of exogenous RNA without apparent destruction. Our autoradiographic analyses show that rat liver C^{14}-RNA is mainly found in the cytoplasm and in the nucleoli of the salivary gland cells of *Dipteran* larvae.

Zusammenfassung. Autoradiographisch wurde festgestellt, dass sich injizierte DNS mit der DNS der polytonen Chromosomen verbindet und in die Zellkerne der Speicheldrüse eingebaut wird.

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Presence and Adaptive Changes of Citrate Cleavage Enzyme in the Yeast *Rhodotorula gracilis*[1]

Citrate cleavage enzyme[2] (ATP citrate lyase: E.C. 4.1.3.8) is widely assumed to be a first regulator enzyme of the fat storage sequence from citrate in animal tissues, where its level depends on the nutritional state of the organism[3]. This communication deals with the presence of a citrate cleavage enzyme also in a yeast species and with its long-term changes in response to different external carbon sources. The yeast *Rhodotorula gracilis* is an obligate aerobe fat-storing organism, which in adequate conditions can develop a true 'obesity'[4]. In the environmental conditions of fat storage a high level of citrate cleavage enzyme has been observed, and this suggests an interpretation of function and regulation of this enzyme in some yeast cells analogous to that proposed for animal cells.

Experimental. The organism used was a strain *Pan* of *Rhodotorula gracilis*, obtained from the Istituto di Microbiologia Agraria of the University of Milan. From the same collection were also obtained other yeast strains of *Saccharomyces cerevisiae* and *Candida utilis*. Yeasts were cultivated in a liquid synthetic medium having the following composition, per liter: $(NH_4)_2SO_4$, 15 mmoles; K_2HPO_4, 5.7 mmoles; NaCl, 8.6 mmoles; $MgSO_4$, 4 mmoles; $CaCl_2$, 2.3 mmoles; $FeCl_3$, 0.018 mmoles; Ca pantothenate, 5 mg; thiamine-HCl, 5 mg; glucose or other carbon sources, as indicated in the Tables and in the Figure; final pH was 4.5. Such a medium, rich in sugar, was described by LUNDIN[5] as particularly suitable to obtain a 'fat-yeast': extensive fat depots are microscopically visible in *Rhodotorula gracilis* cells after cultivation under these conditions in presence of 20–200 m*M* glucose.

Growth took place in well aerated flasks with eccentric agitation, at 30 °C. For the larger cultures a New Brunswick Microferm Fermentor was used.

For enzyme determinations cells were collected by centrifugation and disrupted in a Braun glass-beads homogenizer (5 g of beads per g of yeast; 30 sec at 70 rev/sec) using as suspending medium 100 m*M* potassium phosphate + 10 m*M* 2-mercaptoethanol + 10 m*M* $MgCl_2$, at pH 7.0. The suspension was held 30 min at 4 °C with stirring and then centrifuged at 20,000 *g* for 30 min to obtain a clear extract.

A partial purification of citrate cleavage enzyme was achieved by precipitation with $(NH_4)_2SO_4$ (fraction 0.7–1.6*M*, pH 6.8) and passage through a Sepharose 4B column. Fractions after gel filtration step contained minimal or no malate dehydrogenase activity. Stability of enzyme preparations depends very strongly on pH, optimal conservation being obtained at pH's between 6.5 and 7.0.

Identification and activity measurements of citrate cleavage enzyme were carried out in reaction mixtures having the following basic composition: 200 m*M* Tris-HCl buffer, 10 m*M* $MgSO_4$, 10 m*M* glutathione-SH, 10 m*M* sodium citrate, 0.2 m*M* CoA-SH, 5 m*M* ATP, 0.2 m*M* NADH, 3 IU cryst. malate dehydrogenase, 20–50 µl/ml enzyme preparation. Oxalacetate formation was followed by continuous monitoring of optical extinction change at 366 nm in an Eppendorf recording photometer, thermoregulated at 30 °C. Routine tests were carried out at pH 8.4 and started with ATP.

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In addition to oxalacetate, acetyl-CoA was also identified and dosed as a reaction product in some experiments. For this purpose 0.35 *M* hydroxylamine, adjusted to pH 8.4, was added as trapping agent to the reaction mixture and the acetyl-hydroxamate formed after 10 and 20 min at 30 °C was identified by thin-layer chromatography or dosed with the $FeCl_3$ test. Thin-layer chromatography on silica gel was carried out by using as solvent water-saturated *n*-butanol[6]; acetyl-hydroxamate standards, obtained by treating with NH_2OH acetic anhydride, were run in parallel, and the spots localized by acid $FeCl_3$ coloration (Rf = 0.65). For quantitative determination of acetyl-hydroxamate in the reaction mixture, the procedure of LIPMANN and TUTTLE[7] was applied.

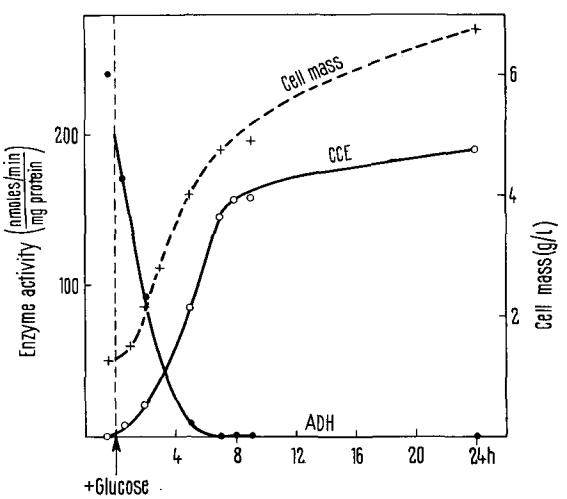
Activity measurements of alcohol dehydrogenase (E.C. 1.1.1.1) were carried out by continuous optical method according to BÜCHER et al.[8]. Total proteins were determined by a biuret method, according to BEISENHERZ et al.[9].

Results. Rhodotorula gracilis, grown in a glucose/ammonium medium, contains a significant citrate cleavage enzyme activity (Tables I and II).

Initial enzyme characterization, carried out on the partially purified preparation, has shown that it requires as cofactor Mg^{++} or Mn^{++}. Optimum pH is 8.4–8.8. The affinity is about 40-fold higher for CoA than for citrate or ATP: affinity for Mn^{++} is higher than for Mg^{++}, but Mn^{++} concentrations above 5 m*M* are inhibitory. The enzyme is inhibited by hydroxylamine.

The presence of citrate cleavage enzyme is not ubiquitous in yeasts: other yeast species, like *Saccharomyces cerevisiae* and *Candida utilis*, grown under the same conditions as *Rhodotorula gracilis*, show no activity (Table I). For comparison, distribution of a well-known yeast enzyme, alcohol dehydrogenase, is also shown in the Table.

Citrate cleavage enzyme in *Rhodotorula* exhibits adaptive properties. Experiments were carried out by determining the changes of enzyme level when the organism was grown 24 h on different carbon sources: alcohol dehydrogenase changes were also determined. Results are reported in Table II.



Time course of adjustment of citrate cleavage enzyme (CCE) and alcohol dehydrogenase (ADH) following addition (arrow) of glucose, at a 200 m*M* concentration, to an ethanol-grown culture of *Rhodotorula gracilis*. Dashed line indicates the growth. Left ordinates: specific enzyme activity (nmoles/min/mg protein). Right ordinates: cell mass (wet weight g/l). Abscissae: time (h).

The time course of enzyme adjustment when glucose is added to a *Rhodotorula* culture grown on ethanol is reported in the Figure. The formation of citrate cleavage enzyme is completely blocked by 75 μM cycloheximide, showing its dependence on undisturbed protein synthesis.

Alcohol dehydrogenase, that is practically lacking in acetate-grown *Rhodotorula glutinis*[10], is formed by *Rhodotorula gracilis* growing on ethanol (Table II): the maximum enzyme level depends on a number of factors, including the Zn content of culture. This enzyme shows an evident glucose repression, as already observed for other yeast cells[11], and even an inactivation rapid and complete under the adopted culture conditions, with no Zn added (see Figure).

Table I. Specific activity of citrate cleavage enzyme (CCE) and alcohol dehydrogenase (ADH) in different yeasts, grown on 20 m*M* glucose as carbon source

Yeast	Enzyme specific activity (nmoles/min/mg protein) CCE	ADH
Rhodotorula gracilis	190	0
Candida utilis	0	100
Saccharomyces cerevisiae	0	280

Cells cultivated aerobically 24 h at 30 °C.

Table II. Specific activity of citrate cleavage enzyme (CCE) and alcohol dehydrogenase (ADH) in *Rhodotorula gracilis* grown on different carbon sources

Carbon source	Initial concentration in the medium (m*M*)	Enzyme specific activity (nmoles/min/mg protein) CCE	ADH
Glucose	200	194	0
Glucose	20	186	0
Citrate	20	170	0
Succinate	30	180	0
Glycerol	400	90	12
Ethanol	60	8	43
Acetate, Glycolate, Glyoxylate	60	(No growth)	

Cells cultivated aerobically 24 h at 30 °C.

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Discussion. The presence or absence of citrate cleavage enzyme in the yeast strains investigated appears as a relevant component in the determination of a diverse 'metabolic structure' of *Rhodotorula* cell with respect to other yeast cells. Endowment with a citrate-utilizing system for fatty acid synthesis would point to some analogy of *Rhodotorula gracilis* with a mammalian liver cell, and the finding of a higher level of citrate cleavage enzyme in the livers of an obese strain of mice, with respect to non-obese animals[3], makes the analogy more suggestive.

The formation of citrate cleavage enzyme is stimulated by glucose and by metabolites of tricarboxylic acid cycle: a concurrent behaviour was found for hepatic enzyme, when the nutritional state was modified in animals[3,12].

The present data do not allow conclusions as to a strict parallelism between changes of citrate cleavage enzyme and short-term control of lipogenesis in *Rhodotorula gracilis*. But the presence of this enzyme in a yeast endowed with a peculiar fat-storing capacity, and the stimulation of its formation under conditions of enhanced lipid accumulation, support the concept of a long-term relationship between the level of citrate cleavage enzyme and fat storage processes.

Riassunto. Nel lievito *Rhodotorula gracilis*, capace di un intenso accumulo lipidico, é stata trovata una ATP citrato liasi, il cui livello cellulare varia a seconda della fonte di carbonio impiegata: il livello é alto su glucosio o intermedi del ciclo degli acidi tricarbossilici, é praticamente nullo su etanolo. L'enzima non é stato trovato in altri lieviti saggiati.

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Selective Incorporation of Zinc into Rat Mast Cells[1]

The joint occurrence of histamine and heparin in mast-cell granules[2,3] as well as the concomitant liberation of heparin and histamine in the dog, guinea-pig and rat[4–10] has suggested the presence of a salt linkage between these agents[11–14]. However, the affinity between heparin and histamine has been found to be too weak to keep the complex intact[11–13]. Histochemical studies have indicated that zinc might be a normal constituent of basophilic leukocytes and mast-cell granules[13–16]. On the basis of in vitro studies showing that the binding of histamine by heparin is greatly increased and stabilized by zinc, KERP[13,14] has postulated the occurrence of ternary heparin-zinc-histamine complexes in mast cells. More recently, the zinc content of isolated rat mast cells was estimated by atomic absorption spectrophotometry and found to be 2.0 μg Zn/10^6 mast cells[17,18]. These data indicate that the zinc content of rat mast cells is at least 40 times higher than that of other cell types present in the rat peritoneal cavity[18], and that zinc is a constituent of the mast-cell granule[17]. The present study employs radioactive zinc (Zn^{65}) as a tracer in order to determine the incorporation of zinc into tissue mast cells as well as various other tissues.

In a first series of experiments, untreated male colony-bred Osborne-Mendel rats (200–240 g) were injected i.p. with 0.5 ml physiological saline containing 3 μC of carrier-free Zn^{65} as zinc chloride (specific activity 500 mc/g Zn; The Radiochemical Centre, Amersham). The rats were killed 48 h later and the various organs removed. Cells were obtained by pressing the organs through a 60-mesh stainless steel screen into saline. The cells were washed 3 times in saline, counted and made up to a final concentration of 10^9 cells/ml. Peritoneal cells and isolated peritoneal mast cells (92–95% mast cells) were obtained as described earlier[3,19] and processed as other cells; mast-cell concentrations were between 2.6 and 6.5×10^6 cells. The suspensions were dried, and radioactivity was measured in a gas flow counter. Results of a typical experiment are shown in the Table. The data show that significant radioactivity could only be detected in peritoneal cell populations containing a relatively high percentage of mast cells. Isolated peritoneal mast cells contained the greatest amount of radioactivity. Since the number of mast cells in each experiment was considerably lower than the number chosen for other cell types, radioactivity of the latter was determined additionally in samples containing the same number of cells as present in mast-cell preparations. Under these conditions, no radioactivity was detectable in all the other cell types examined. In a further series of experiments, rats were injected with 3 μC Zn^{65} i.m., and similar results were obtained.

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