*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 $6.5 \times 10^6$ 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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Distribution of $Zn^{65}$ 48 h following i.p. injection

| Tissue | Cpm per $2.6 \times 10^6$ cells[a] | | Cpm per $10^9$ cells[a] |
|---|---|---|---|
| Mesenterial | | 0 | 22.5 |
| lymph nodes | | 0 | 12.9 |
| | | 0 | 12.0 |
| Thymus | | 0 | 24 |
| | | 0 | 15 |
| | | 0 | 14 |
| Spleen | | 0 | 18 |
| | | 0 | 29 |
| | | 0 | 18 |
| Liver | | 0 | 76 |
| | | 0 | 53 |
| | | 0 | 42 |
| Pancreas | | 0 | 22 |
| | | 0 | 21 |
| | | 0 | 39 |
| Lungs | | 0 | 21 |
| | | 0 | 30 |
| | | 0 | 28 |
| Peritoneal cells (mast cells) | $0.011 \times 10^9$[b] ($0.5 \times 10^6$)[c] | 70 | 6,400 |
| | $0.013 \times 10^9$[b] ($0.7 \times 10^6$)[c] | 69 | 5,300 |
| | $0.014 \times 10^9$[b] ($0.7 \times 10^6$)[c] | 68 | 4,800 |
| Isolated mast cells | | 38 | 14,600 |

[a] The values are corrected for background. [b] Total number of peritoneal cells. [c] Number of peritoneal mast cells in brackets.

To find out whether zinc is released from mast cells under conditions known to cause a release of histamine, rats which had been given 50 μC $Zn^{65}$ i.p. 48 h earlier, were treated with the chemical histamine-liberator, compound 48/80 (400 μg/animal i.p.); other animals received phosphate-buffered saline i.p. The peritoneal fluid was harvested 15 min later, and radioactivity of dried supernatants measured. In controls ($672 \pm 350$ cpm), radioactivity was found to be clearly lower than in animals treated with compound 48/80 ($2908 \pm 663$). The present results suggest that radioactive zinc is incorporated selectively into rat tissue mast cells. And significant amounts of zinc were released by the chemical histamine-liberator, compound 48/80. However, the mechanism of binding of zinc to mast-cell granules, and its role and mode of mobilization in pathophysiological processes needs further elucidation.

*Zusammenfassung.* Bei der Albinoratte findet sich 48 h nach parenteraler Verabreichung von radioaktivem Zink ($Zn^{65}$) ein hoher Anteil der Aktivität in den Gewebemastzellen. Nach Verabreichung des chemischen Histaminliberators, Compound 48/80, wird ein erheblicher Teil von $Zn^{65}$ aus den Zellen frei.

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## Collagenolytic Enzymes in Human Serum

The search for collagenases in the tissue of higher animals has led to the isolation of several proteases with acidic and neutral pH optima[1]. Gries and Lindner[2,3] could demonstrate that various mammalian tissues contain enzymes that are capable of splitting soluble peptides from native insoluble collagen under physiological conditions. Animal collagenases that have been shown to cleave the collagen molecule into 2 pieces, representing three-quarters and one-quarter of the molecule, were found some years ago for the first time by Gross et al.[4] and later by Fullmer et al.[5], Evanson et al.[6], Lazarus et al.[7], and Eisen et al.[8].

A new class of collagenolytic enzymes, which are different from the enzymes mentioned above, and which resemble bacterial collagenases in their specificity for the apolar regions of the collagen molecule, have recently been demonstrated by Strauch et al.[9–11] in living cells of higher animals and in invasion zones of human tumuors[12]. The activity of these enzymes was measured by means of the synthetic substrate PZ-Pro-Leu-Gly-Pro-D-Arg (PZ = *p*-phenylazobenzyloxycarbonyl-), recently developed by Wünsch and Heidrich[13].

This substrate, which has a sequence analogous to the repeating sequence -Gly-Pro-X-Gly-Pro-X- (Pro is proline or hydroxyproline and X a variable amino acid) present in the apolar regions of collagen molecule, is split by a collagenase of the bacterial type between the Leu- and Gly-residues. The fragment PZ-Pro-Leu is insoluble in aqueous solutions at acid pH and can be extracted from the reaction mixture with organic solvents and determined spectrophotometrically. The C-terminal arginine makes the peptide easily soluble in water and the D-form makes the compound stable against attack by trypsin, carboxypeptidase and other cell proteases. The substrate was found to be non-toxic for the cells at the concentrations required for the determination. The method is very sensitive and allows the measurement of extremely low collagenolytic activity.

The isolation, the mode of action, and the properties of animal collagenases with the specificity for apolar regions of the collagen molecule will be published else-

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