liver pyruvate carboxylase activity is elevated in fasted and in alloxan diabetic rats and after administration of corticosteroids; these findings are consistent with a role for this enzyme in gluconeogenesis.

The presence of the phosphoenolpyruvate carboxykinase has been previously demonstrated in cat, rat, guinea-pig and ox brain cortex mitochondria[14].

The very high similarity of some characteristics of the cortex and liver pyruvate carboxylase and the common intramitochondrial location of the brain cortex pyruvate carboxylase and phosphoenolpyruvate carboxykinase support the hypothesis that the 2 enzymes, functioning in sequence, may catalyse reactions constituting a pathway for phosphoenolpyruvate synthesis from pyruvate or lactate in mammalian brain cortex mitochondria.

Riassunto. La presenza della piruvico carbossilasi è stata evidenziata nella corteccia cerebrale di ratto, cavia, coniglio, gatto, bue. È stata studiata la localizzazione subcellulare della piruvico carbossilasi cerebrale; l'enzima risulta essere particolato: il citoplasma solubile non presenta alcuna attività mentre i mitocondri pesanti costituiscono la frazione più attiva. Viene discusso il possibile ruolo fisiologico della piruvicocarbossilasi nella corteccia di mammifero.

R. A. Felicioli, F. Gabrielli, and C. A. Rossi

Istituto di Chimica Biologica dell'Università di Pisa (Italy), June 6, 1966.

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Production of 4-Imidazole Ethanol from Histamine by *Saccharomyces rouxii*[1]

Histamine is converted in mammalian tissue by diamine oxidase to 4-imidazole acetaldehyde. Xanthine oxidase or aldehyde dehydrogenase converts this to 4-imidazole acetic acid[2], which is found in the urine[3]. Nakajima and Sano[4] found that the urine of patients treated with an aldehyde dehydrogenase inhibitor contained small amounts of 4-imidazole ethanol. They also found traces of this compound in the urine of normal subjects. The concentration is low; we calculate their results to indicate an excretion by normal man of less than 200 µg/day. Such low amounts suggest that the study of its formation in animal tissue will be difficult.

In a study of histidine metabolism by yeasts[5] we have found several strains which produce imidazole ethanol from histamine in readily detectable amounts, and one strain which produces this compound as the major product of histamine degradation. This paper describes the identification and estimation of 4-imidazole ethanol produced by *Saccharomyces rouxii*.

Methods. Imidazole ethanol was prepared as the chloroplatinate, as described by Wrede and Holtz[6]. *S. rouxii* PRL 411–64 was isolated from a bumblebee's nest from Melfort, Saskatchewan.

The growth medium used was Yeast Carbon Base (Difco) containing 3 m*M* histamine or 3 m*M* L-histidine. Yeasts were grown aerobically in 125 ml shake flasks on a rotary shaker at 150 rpm at 25 °C. The substrates were completely metabolized after 3 days. The yeast cells were removed by centrifugation. 2 or 10 µl of supernatant were applied to Whatman No. 1 paper, chromatographed in 8 solvents, and imidazole derivatives detected with diazotized sulphanilic acid (DAS) and sodium carbonate[7]. The Rf of the product was compared with those of imidazole ethanol and imidazole acetic acid.

The amount of imidazole ethanol in 0.02 ml of supernatant was determined by the formation of a coloured compound with diazotized *p*-nitro aniline[8]. The optical density at 550 nm was measured with a Beckman DU Spectrophotometer.

Results and discussion. The only detectable product of histidine metabolism had the same Rf as imidazole acetic acid. When 2 µl of supernatant from the culture grown on histamine was chromatographed as described above, only one imidazole derivative reacting with DAS could be observed. The Rf values of this product in the 8 solvents were indistinguishable from those of authentic imidazole ethanol. When 10 µl of sample were chromatographed, a faint spot with the same Rf as imidazole acetic acid was also detected.

Chromatographic identification of imidazole-4-ethanol

Solvent	Rf · 100	
	Imidazole-4-ethanol	Histamine product
n-Propanol-N-acetic acid, 3:1	52	54
Butanol-acetic acid-pyridine-H_2O, 4:1:1:2	28	30
Methanol-*n*-butanol-benzene-H_2O, 2:1:1:1	88	87
Butanol-acetic acid-H_2O, 4:1:1	35	34
Butanol-pyridine-H_2O, 1:1:1	75	75
Ethanol-H_2O, 77:23	73	71
iso-Propanol-conc. NH_4OH-H_2O, 80:5:15	88	86
tert-Butanol-formic acid-H_2O, 70:15:15	64	63

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The colorimetric determination of imidazole ethanol indicated a concentration of 2.6 ± 0.4 m*M*. This would represent a conversion of approximately 87%. However, TABOR[8] has pointed out that the diazotized *p*-nitro aniline method is subject to interference from many biological materials, which may enhance or inhibit the colour development. He states the accuracy of a determination may be ± 10–20%. Moreover, in our experiment, a slight amount of colour probably arose from the imidazole acetic acid present in the sample. Therefore, the estimated concentration of imidazole ethanol should not be considered as accurate. However, it does indicate that most of the histamine was converted to imidazole ethanol.

ERLICH[9,10] reported the formation of unstated amounts of imidazole ethanol by yeast fermenting histidine. However, a strain of *S. cerevisiae* Sc6 in our experiment failed to produce imidazole ethanol from histidine or histamine, aerobically or anaerobically.

NAKAJIMA and SANA[4] suggest that imidazole ethanol may be formed from imidazole acetaldehyde by an alcohol dehydrogenase. If *S. rouxii* has similar pathways for histamine degradation, our results suggest that in the yeast the activity of the alcohol dehydrogenase is much higher than that of the aldehyde oxidase.

Zusammenfassung. *Saccharomyces rouxii* PRL-411-64 wurde im Nährmedium mit Histamin als Stickstoffsubstrat kultiviert. Das Hauptprodukt des Metabolismus wurde papierchromatographisch als Imidazol-4-ethanol identifiziert.

T. LARUE and J. F. T. SPENCER

National Research Council of Canada, Prairie Regional Laboratory, Saskatoon (Saskatchewan, Canada),
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Quantitative und qualitative Untersuchungen der Esteraseaktivität in normalen Granulozyten, normalen Lymphozyten und in leukämischen Zellen[1]

Die Esteraseaktivität wurde vorwiegend anhand histochemischer Methoden studiert[2–7]. Bis heute beschäftigten sich nur wenige Autoren mit quantitativen biochemischen Untersuchungen[8,9]. Eine qualitative elektrophoretische Analyse der Leukozytenproteine mit Esteraseaktivität wurde nur von ANDERSEN und SÖLVSTEN[10] ausgeführt. Sowohl die quantitativen biochemischen, wie auch die qualitativen elektrophoretischen Bestimmungen wurden jedoch mit heterogenen Leukozytenpräparationen durchgeführt.

In dieser Arbeit berichten wir über die Ergebnisse der quantitativen und qualitativen Untersuchungen der Esteraseaktivität in Präparaten von normalen menschlichen Granulozyten, respektive Lymphozyten, mit einer Reinheit von über 90%, und in leukämischen Zellpräparaten nach kompletter Entfernung der reifen Granulozytenkomponente.

Material und Methoden. Die Granulozyten wurden nach der Methode von DIOGUARDI et al.[11] isoliert. Die Präparationen von Lymphozyten und leukämischen Myelozyten erfolgten nach der Methode von AGOSTONI und IDEO[12]. Die quantitative Bestimmung der Esteraseaktivität wurde nach der Methode von HARDIN et al.[8] ausgeführt. Die Charakterisierung der Esterasen erzielte man durch Agargelelektrophorese, auf Platten (15 cm lang, 4 mm dick) von 1% Gel, getränkt in Na-Veronalpuffer pH 8,2, Ionenstärke 0,05. Die Dauer der Elektrophorese betrug 2 h, mit einer Potentialdifferenz von 100 V. Zur Sichtbarmachung der Esteraseaktivität benützte man β-Naphthylacetat (Mann Res. Lab. Inc.) und Ortho-Dianisidin-Tetrazoniumsalz (Sigma) in einem Phosphatpuffer pH 7,4. Die Platten wurden in dieser Lösung während 6 h in einem Trockenschrank bei 37°C inkubiert, anschliessend in 2%iger Essigsäure gewaschen und im Trockenschrank bei 37°C getrocknet. An Stelle von β-Naphthylamin wurden andere Substrate, wie zum Beispiel Indoxylacetat und Naphthol-AS-D-Chloracetat verwendet; es wurden damit jedoch keine befriedigenden Resultate erzielt.

Ergebnisse und Diskussion. Die Esteraseaktivität zeigt, gegenüber den normalen Granulozyten, einen signifikanten Anstieg in den unreifen Zellen der myeloischen Leukämie (Tabelle). Gleich verhalten sich die lymphatischen Leukämiezellen gegenüber den normalen Lymphozyten (Tabelle). Im besonderen glauben wir unterstreichen zu können, dass diejenigen Fälle, welche Zellen mit geringerem Reifegrad enthielten, die höchsten Werte an Esteraseaktivität aufwiesen. Dies gilt sowohl für die myeloische, wie für die lymphatische Serie. Ein Anstieg der Esteraseaktivität in den Leukämiezellen wurde schon von FREI et al.[9] beobachtet, welcher, da er nicht über reine Zellpräparate verfügte, das Problem mathematisch zu lösen versuchte.

Die elektrophoretische Analyse zeigte in normalen Granulozyten, wie in normalen Lymphozyten vier Zonen

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