lytic waves (Figure 2, curve h). From the height of the F3 fraction wave it may be deduced that it contains a slight amount of cysteine.

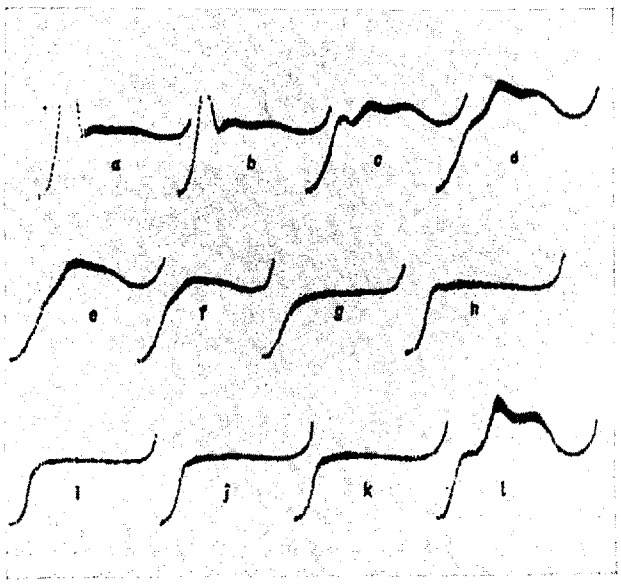

Fig. 2. Polarographic curves of the chicken erythrocyte histones. Curves a–g: WH (20, 30, 50, 100, 150, 200 and 2500 μg/ml); curve h: WH oxidized with performic acid (100 μg/ml); curve i: F_1A histone (60 μg/ml); curve j: F_1B histone (50 μg/ml); curve k: F_2 histone (58 μg/ml); curve l: F_3 histone (45 μg/ml).

The decrease, deformation or disappearance of the catalytic waves of histones at increased protein concentration (Figure 2, curves e–h) may be explained by the aggregation of the histone molecules at basic pH[14]. Therefore, formation of Co^{3+}-protein complexes causing the polarographic activity is met with spatial difficulties. This phenomenon may explain the fact that polarographically HAMER[15] did not find any cysteine in calf thymus histone.

Zusammenfassung. Das Erythrocytenhiston des Hühnchens zeigt eine polarographische Aktivität, die für das Vorhandensein von SH-Gruppen spricht. Extrahiertes Gesamthiston wurde an einer Carboxymethylcellulosesäule chromatographiert. Von den erhaltenen 4 Fraktionen enthielt nur eine (F3) polarographisch nachweisbares Cystein.

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Formate Oxidation in *Saccharomyces cerevisiae*

Oxidation of formate to carbon dioxide is a significant pathway in a variety of animal and plant tissues. Evidence has been presented by CHANCE[1] and others[2,3] that in animal tissues the peroxide-catalase complex is responsible for this oxidation. In the case of microorganisms, diverse mechanisms, such as a true oxidase in *Aspergillus niger*[4] and cytochrome-specific dehydrogenase in *Escherichia coli*[5] and *Nitrobacter agilis*[6], have been shown to be operative.

A *de novo* synthesis of catalase on aeration of anaerobically grown cells of *Saccharomyces cerevisiae* has been reported earlier[7,8]. Formate oxidation in such a system has now been studied to examine the possibility of a catalase-dependent reaction in this organism.

A locally isolated strain of *Saccharomyces cerevisiae* was grown on a medium consisting of glucose 2%, Bactopeptone 1%, yeast extract 0.2% for 20 h at 30°C. Anaerobic condition was ensured by using 500 ml conical flasks filled to the neck with the medium. The cells were then harvested by centrifugation and were suspended in a fresh non-growth medium of composition glucose 1%, potassium dihydrogen phosphate 1%, magnesium sulphate 0.01% and calcium chloride 0.01%. This suspension was divided into 10 ml portions and was aerated in 50 ml conical flasks by shaking in a reciprocating shaker for different periods, after which the cells were separated, washed with distilled water and used for the assay of catalase activity and formate oxidation. Catalase activity was estimated by a modified titanium colour reaction method[9] and is expressed as *Katf*, where

$$Katf = (\ln X_0/X_t)/et \cdot 10^2$$

in which X_0 and X_t are the residual hydrogen peroxide at '0' and 't' minutes and 'e' the concentration of the enzyme source. The extent of formate oxidation was followed by incubating the cells in standard Warburg respirometer vessels at 37°C for 15 min with Krebs-Ringer phosphate buffer pH 7.4, 0.1 M and formate-C^{14} ($1.4 \cdot 10^4$ cpm, Radiochemical Centre, Amersham, England) to a total volume of 3 ml. The respired carbon

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dioxide was trapped in 0.2 ml of 10% potassium hydroxide contained in the central well and was precipitated as barium carbonate, transferred to counting planchets for the assay of radioactivity in Tracerlab SC-16 windowless gas-flow counter in conjunction with SC-51 autoscaler.

Effect of aeration on catalase induction and formate oxidation in *Saccharomyces cerevisiae*

Period of aeration h	Catalase activity Units/ml	Carbon dioxide activity cpm
0	34	117
1	138	566
2	182	811
4	246	1131
4[a]	43	212

[a] With addition of potassium cyanide (0.01 *M*).

It may be seen from the Table that cells of *Saccharomyces cerevisiae* grown anaerobically are practically devoid of catalase activity, and such cells also oxidize labelled formate only to a small extent. Aeration of these cells for 1, 2 and 4 h leads to gradual increase in catalase activity accompanied by increase in oxidation of formate. Besides, the addition of potassium cyanide (0.01 *M*) to incubation medium containing cells aerated for 4 h, inhibits the oxidation of the substrate almost completely. These findings indicate a catalase-dependent mechanism of formate oxidation in yeast.

Résumé. La synthèse, *de novo*, de la catalase pendant l'aération des cellules de *Saccharomyces cerevisiae* qu'on a fait croître préalablement en l'absence d'oxygène est accompagnée d'une capacité augmentée pour l'oxidation du formiate marqué. Le cyanure de potassium inhibe l'activité catalasique aussi bien que l'oxidation du formiate.

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Biosynthesis of Alkaloids. On the Occurrence of Keto Acids in *Papaver somniferum* L. Plants

The biosynthesis of opium alkaloids from the amino acid, tyrosine, has been proved by feeding experiments using labelled tyrosine[1-4]. The sequence of reactions leading from amino acids or their biochemical equivalents to alkaloids of the papaverine or morphine type is believed to contain deaminated and decarboxylated derivatives of tyrosine[5,6]. The present state of knowledge of alkaloid biosynthesis[7,8] demonstrates the general importance of keto acids in this process.

The occurrence of keto acids has been investigated in plants of *Papaver somniferum* L. at various stages of their development by means of paper chromatography of their 2,4-dinitrophenylhydrazones. The plants were treated according to [9] and [10] and the 2,4-dinitrophenylhydrazones obtained were characterized by means of paper chromatography using *n*-butanol-ethanol-0,5*N*-NH_3 (7:1:2)[11]. In all the ontogenetic stages studied pyruvic, α-ketoglutaric and oxaloacetic acids were found in varying amounts. At the stages immediately before and after flowering, traces of substances were found which might correspond to the 2,4-dinitrophenylhydrazones of some aromatic keto acids. In later experiments we succeeded to demonstrate the presence of phenylpyruvic and *p*-hydroxyphenylpyruvic acid. The 2,4-dinitrophenylhydrazones, obtained by treating a large amount of material (677 g and 954 g of fresh plants respectively) from the above mentioned stages, were separated by means of paper chromatography using the system *n*-butanol-3% NH_3 (1:1) for the first run, and veronal-acetate buffer solution (pH 8,6) for the second direction on Whatman No. 3[12]; their identity was proved by treating the standard keto acids in a similar way. In addition, the 2,4-dinitrophenylhydrazones were converted to the corresponding amino acids by reduction according to [13]. High concentrations of aspartic and glutamic acid and of alanine interfered in chromatographic analysis. Aromatic amino acids were therefore separated by adsorption on active carbon (Sutcliffe, Speakman and Co. Ltd., Leigh, Lancashire) activated according to [14] and, after washing with water, were eluted by 3-fold extraction with 20% acetic acid with 5% phenol; after removing phenol and dinitro-aniline by ether, the amino acids were bound on Dowex 50 × 8, eluted with ammonia and the solution analysed. It is thus possible to determine the presence of phenylalanine and tyrosine in the presence of a 500-fold excess of aliphatic amino acids. A mixture of the 2,4-dinitrophenylhydrazones from poppy plants, treated in the manner described, gives spots corresponding to phenylalanine and tyrosine after paper chromatography using the *n*-butanol-acetic acid-water (4:1:5) system. This fact proves the presence of phenylpyruvic and *p*-hydroxyphenylpyruvic acid in *Papaver somniferum* plants at the above-mentioned stages.

The observation of the aromatic keto acids in poppy plants is in line with present-day opinions on the mecha-

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