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.

S. VENKATARAMAN and A. SREENIVASAN

Central Food Technological Research Institute, Mysore (India), January 14, 1964.

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 im-Portance 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 8 and 10 and the 2,4-dinitrophenylhydrazones obtained were characterized by means of paper chromatography using n-butanol-ethanol-0, $5N-NH_3$ (7:1:2) 11. In all the ontogenetic stages studied pyruvic, a-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% NH₃(1:1) for the first run, and veronal-acetate buffer solution (pH 8,6) for the second direction on Whatman No. 312; 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-

- ¹ A. R. Battersby and B. J. T. Harper, Chem. and Ind. 1958, 363. ² E. LEETE, Chem. and Ind. 1958, 977; J. Am. chem. Soc. 81, 3948 (1960).
- ³ G. Kleinschmidt and K. Mothes, Z. Naturforsch. 14b, 52 (1959).
- ⁴ A. R. BATTERSBY and R. BINKS, Proc. chem. Soc. 287, 360 (1960).
- 5 A. R. Battersby and B. J. T. Harper, J. chem. Soc. 1962, 3526.
- ⁶ A. R. Battersby, R. Binks, and B. J. T. Harper, J. chem. Soc. 1962, 3534.
- ⁷ K. Mothes, Pharmazie 14, 122, 178 (1959).
- ⁸ K. Mothes and H. R. Schutte, Angew. Chem. int. Edit. 2, 442 (1963).
- ⁹ D. CAVALLINI and N. FRONTALI, Biochem. biophys. Acta 13, 439 (1954).
- 10 A. I. VIRTANEN, J. K. MIETTINEN, and H. KUNTTU, Acta chem.
- scand. 7, 38 (1953). 11 M. F. S. El Hawary and R. H. S. Thompson, Biochem. J. 53, 340 (1953).
- 12 R. POHLOUDEK-FABINI and H. WOLLMANN, Pharmazie 17, 5
- ¹³ M. Alfthan and A. I. Virtanen, Acta chem. scand. 9, 186 (1955).
- ¹⁴ S. M. Partridge, Biochem. J. 44, 521 (1949).

nism of biosynthesis of aromatic amino acids in microorganisms 15 . The precise location of phenylpyruvic and p-hydroxyphenylpyruvic acid in the series of biogenetic reactions leading to opium alkaloids remains, however, an open question.

Zusammenfassung. Bei Papaver somniferum L. wurde im Laufe der Ontogenese die Anwesenheit von Ketosäuren des Zitronensäurecyclus verfolgt. Zur Blütezeit und nach dem Verblühen konnten auch die aromatischen Ketosäuren, Phenylbrenztraubensäure und p-Hydroxyphenylbrenztraubensäure nachgewiesen werden (2,4-Di-

nitrophenylhydrazone und nach Reduktion zu Aminosäuren: Phenylalanin und Tyrosin).

A. JINDRA, Z. ŠÍPAL, and V. HUDECOVÁ

Department of Biochemistry and Microbiology, Faculty of Pharmacy, Bratislava, and Department of Biochemistry, Charles University, Praha (Czechoslovakia), January 27, 1964.

15 B. D. Davis, Arch. Biochem. Biophys. 78, 497 (1958).

The Serum Histaminase Activity in Guinea-Pig Pregnancy

The increased Histamine (H) formation and a higher H metabolism in pregnancy of various species has already been reported ¹⁻⁶. The interest in the role of H in pregnancy has increased, especially since Kahlson's hypothesis was published concerning H as an important factor in tissue growth ^{7,8}. But Kahlson's hypothesis, though very interesting, is limited on account of the use of a single species, the rat, in his investigations. That makes any generalization impossible.

More active metabolism of H in pregnancy is manifested, among other things, by the increased histaminase activity. In this paper results are presented concerning the histaminase activity in the guinea-pig in different periods of gestation and one day after parturition.

Methods. Experiments were made on 20 guinea-pigs of 450-620 g body weight. Every two or four weeks, 5 ml of blood were taken from each guinea-pig by heart puncture, and the histaminase activity was determined in the serum. In each guinea-pig we obtained 3 to 5 histaminase activity determinations in different periods of pregnancy. The last determination was made 24 h after parturition. The histaminase activity was determined by KAPELLER-ADLER's microvolumetric method. One PU gives 0.46 μg/g/h of histamine inactivated (6.95·10-6 μmol H/min).

Results. The results are presented in the Figure. The histaminase activity in the guinea-pig begins to increase in the second week of gestation and reaches its peak in about 4-7 weeks. Then the histaminase activity decreases but remains detectable, in every case, before parturition.

24 h after parturition the histaminase activity reaches its normal level. The shape of the curve of histaminase activity in the guinea-pig is almost the same as that in pregnant women $^{9-12}$.

It cannot be excluded that the reasons for augmented histaminase activity are the same. Unfortunately, the H level in pregnant women and pregnant guinea-pigs has not been examined. In pregnant rats the H level is elevated and depends on the number of litters. The higher the number of litters, the higher is the H level 2, 8, 6, 18. Upon removing the foetuses the H level decreases and reaches normal 13. Our findings are the same with regard to the histaminase activity in women after artificial interruption. 24 h after abortion, the histaminase activity reaches its normal level 10.

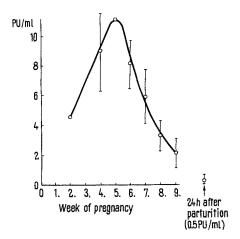
The analogy suggests that augmented histaminase activity is related to a higher H level in pregnancy. If it is taken into account that H production by the rat foetus is very active and H passes to the mother, it seems probable that augmented histaminase activity in maternal blood can be related to that phenomenon.

It is possible that H plays an important role in pregnancy not only in the rat but also in women and in the guinea-pig.

Résumé. Chez les cobayes enceintes l'activité de l'histaminase est considérablement augmentée.

Cz. Maślinski and A. Niedzielski

Department of General and Experimental Pathology, School of Medicine, Lódz (Poland), December 23, 1963.



- C. F. Code and G. A. Hallenbeck, J. Physiol. 159, 66 P (1961).
 G. Kahlson, E. Rosengren, and H. Westling, J. Physiol. 140,
- 12 P (1958).
- ⁸ G. Kahlson, E. Rosengren, and H. Westling, J. Physiol. 143, 91 (1958).
- ⁴ L. Kameswaran and G. B. West, J. Physiol. 160, 564 (1962).
- ⁵ S. E. LINDELL, K. NILSSON, R. W. SCHAYER, and H. WESTLING, J. Physiol. 143, 62 P (1958).
- ⁶ G. B. West, Int. Arch. Allergy 16, 39 (1960).
- ⁷ G. Kahlson, Perspect. Biol. Med. 5, 179 (1962).
- ⁸ G. Kahlson, Proc. Intern. Union Physiol. Sci. XXII Intern. Congr. Leiden 1, 856 (1962).
- 9 R. KAPELLER-ADLER, Biochem. J. 48, 99 (1951).
- 10 Cz. Mašliński, A. Niedzielski, and B. Redziejowska, Gynaecologia, in press (1964).
- 11 H. SWANBERG, Acta physiol. scand. 23, Suppl. 79 (1950).
- ¹² F. Wicksell, Acta physiol. scand. 17, 395 (1949).
- ¹⁸ G. KAHLSON, E. ROSENGREN, H. WESTLING, and T. WHITE, J. Physiol. 144, 337 (1958).