Catalytic Deamination of Asparagine

In a previous note[1] we have shown the decarboxylation of aspartic acid under the catalytic action of copper. The aim of this paper is to show the deamination of asparagine also under the catalytic action of copper.

The optimum concentration of Cu^{++} for the asparagine used was M/4 (Figure 1).

Temperatures ranging between 80 and 100 °C were found to be more favourable for the catalytic action of copper than lower temperatures. At room temperature no deamination takes place.

The influence of physical conditions on the catalysis of deamination was investigated by preparing asparagine solutions (130 mg/100 ml) with (Figure 2) or without

[1] A. Marx, Maria Sendrea and Maria Petcovici, in press.

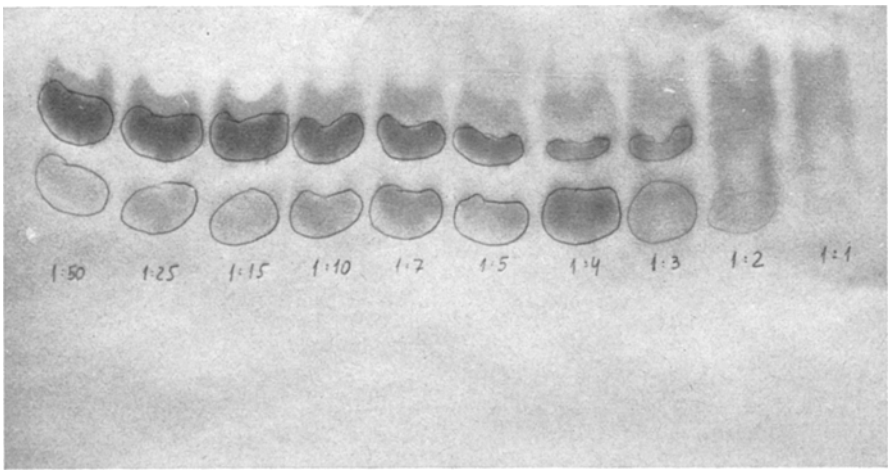

Fig. 1. Chromatography of asparagine solutions dry-evaporated at 100 °C in the presence of $CuSO_4$ at different molarities (compared with that of asparagine). Top row asparagine, below aspartic acid formed.

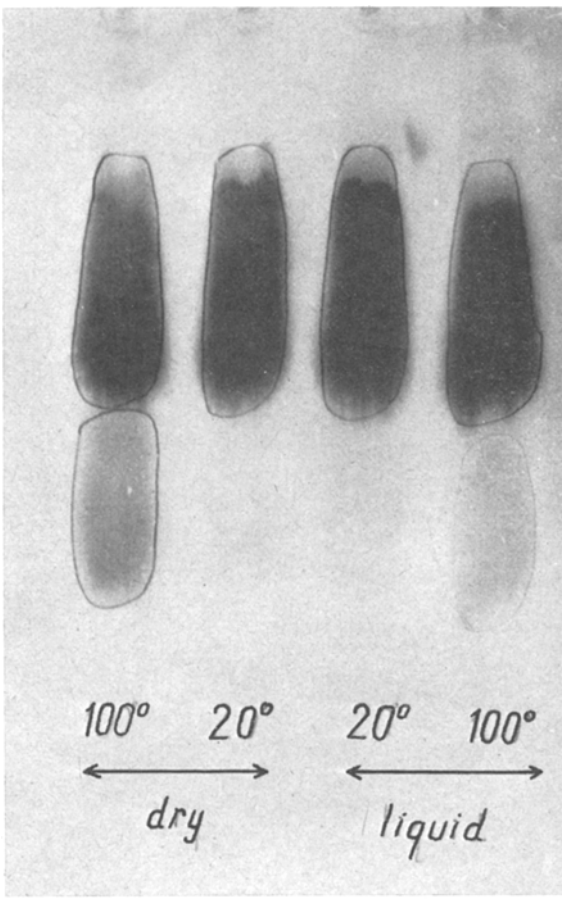

Fig. 2. Chromatography of 130 mg/100 ml asparagine solutions with 62 mg/100 ml $CuSO_4 \cdot 5H_2O$. 2 samples were evaporated to dryness and then maintained for 60 min at 100 or 20 °C, respectively. 2 samples were similarly treated in the liquid state.

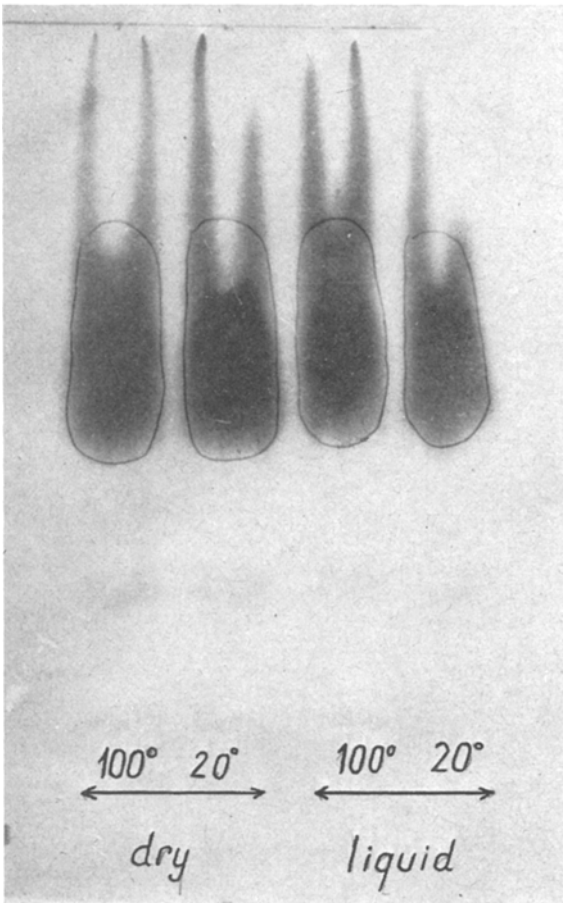

Fig. 3. Chromatography of 130 mg/100 ml asparagine solutions without copper sulphate. 4 samples treated as indicated for Fig. 2.

(Figure 3) the addition of $CuSO_4 \cdot 5H_2O$ (62 mg/100 ml). 10 ml samples were maintained for 60 min at 20 °C or at 100 °C, in the liquid state or in the firm body (after previous drying by evaporation at 20 °C), and then subjected to chromatography, as indicated below. From these chromatograms it may be seen that, at 100 °C, the reaction takes place also in solution but if the reaction mixture is dry-evaporated at 20 °C and then heated up to 100 °C, the formation of aspartic acid is more intense. Hence, catalysis is promoted when heating is carried out in the dry state.

Optimum conditions of deamination were obtained thus: commercial asparagine (containing impurities of aspartic acid) was used after its purification on preparative paper chromatography. 130 mg purified asparagine and 62 mg $CuSO_4 \cdot 5H_2O$ were disolved in 10 ml H_2O. The solution was dry-evaporated at 20 °C and kept for 60 min at 100 °C, then redisolved in 0.5 ml H_2O and applied on Scheleicher-Shüll 2043a chromatographic paper 10×0.01 ml (superposed drops). Eluent *n*-buthanol-acetic acid-H_2O (4:1:5). After 3 days of run, aspartic acid separates from asparagine (tested with ninhydrine). The amount of aspartic acid formed was determined by the method of MOORE et al.[2]. In optimum conditions of catalysis about 20% asparagine is transformed into aspartic acid[3].

Zusammenfassung. Es wird die katalytische Deaminierung des Asparagins mittels Cu^{++} bei 80°–100 °C beschrieben. In diesem Temperaturbereich findet die Reaktion auch in Lösungen statt, während die Katalyse im Trockenrückstand bedeutend stärker ist. Die optimale Konzentration des Cu^{++} (bezogen auf das Asparagin) ist 1:4 *M*. Unter den günstigsten Bedingungen werden 20% des Asparagins zu Asparaginsäure deaminiert.

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Cantacuzino Institute, Bucuresti (Romania), 28 July 1969.

[2] S. MOORE, P. H. SPACKMANN and W. H. STEIN, Anal. Chem. *30*, 1185 (1958).

[3] Acknowledgment. We wish to thank Miss STELA COSTEA and Mr. GH. ANGELESCU for their valuable help in this study.

Suppression of Tryptophan Pyrrolase Induction in Porphyric Animals

In the preceding paper[1] the inhibitory effects of phenylhydrazine (PHZ) on tryptophan pyrrolase (TP) induction by tryptophan was described. These effects of PHZ on TP suggested that the inhibition of TP induction only when the enzyme was induced by tryptophan, not by glucocorticoid, appeared to be due to the disturbance in porphyrin metabolism.

The present paper is concerned with studies of changes in free porphyrins and delta-aminolevulinic acid (ALA) levels in urine of PHZ-treated rats.

Materials and methods. Male rats of Wistar strain weighing 200–250 g were used throughout the present study. All animals used here were adrenalectomized 4 to 6 days before experiments and maintained on 1% sodium chloride solution as drinking water and a commercial diet ad libitum.

Details for the preparation of the enzyme source and assay procedure of TP have previously been described[1]. Determinations of both levels of free porphyrins and ALA in urine were performed by the methods of SCHWARTZ et al.[2] and MARZERALL and GRANICK[3] respectively.

Results. As illustrated in Figure 1, administration of PHZ caused an increase in TP activity in non-induced rats; in contrast, induction of the enzyme by tryptophan began to decrease to approximately 40% of initial level within 24 h, gradually reaching the initial level at 3 days.

On the other hand, Figure 2 showed that injection of PHZ in rats caused significant increase in ALA as well as free porphyrin levels in urine. These changes in urinary components associated with porphyrin metabolism after PHZ treatment seemed to be related to depression of TP induction. In the determination procedure used here uroporphyrin was negligible as indicated by SCHWARTZ et al.[2]

Discussion. With regard to the disturbance in porphyrin metabolism in both human diseases and experimental animals, elevation of urinary free porphyrin levels has been reported by many investigators[4–6], and inhibition of tryptophan-mediated TP induction was also observed in porphyric animals[7].

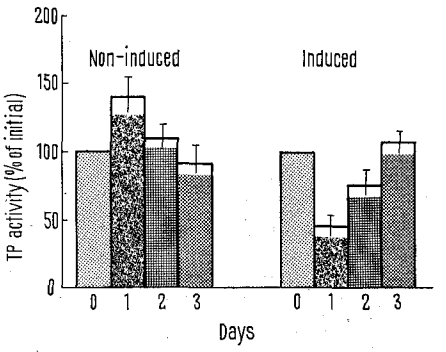

Fig. 1. Changes in induction of tryptophan pyrrolase levels in phenylhydrazine-treated rats. Experimental porphyria was produced by a single injection of phenylhydrazine given on day 0 in a dose of 40 mg/kg i.p. Tryptophan pyrrolase was induced by administration of L-tryptophan in a dose of 500 mg/kg i.p. on 0, first, second and third days after phenylhydrazine treatment. Values presented are means of at least 4 trials and vertical lines indicate standard error of the mean.

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