(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.

A. MARX, MARIA SENDREA and MARIA PETCOVICI

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].

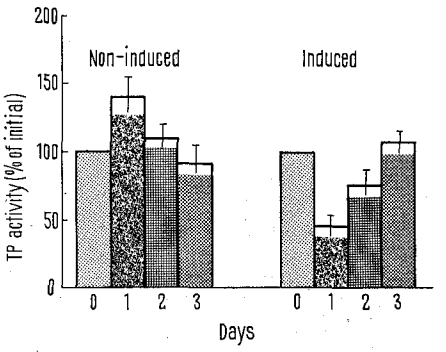

Fig. 1. Changes in induction of tryptophan pyrrolase levels in phenylhydrazine-treated rats. Experimental porphyria was produced by a single injection of phenylhydrazine given on day 0 in a dose of 40 mg/kg i.p. Tryptophan pyrrolase was induced by administration of L-tryptophan in a dose of 500 mg/kg 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.

[1] T. SATOH, Enzymol. Biol. Clin., in press (1969).

[2] S. SCHWARTZ, L. ZIEVE and C. J. WATSON, J. Lab. clin. Med. *37*, 843 (1951).

[3] D. MAUZERALL and S. GRANICK, J. biol. Chem. *219*, 435 (1956).

[4] T. ONO, M. UMEDA and T. SUGIMURA, Gann *47*, 171 (1956).

[5] F. D. MATTEIS and C. RIMINGTON, Br. J. Derm. *75*, 91 (1963).

[6] E. W. HURST and G. E. PAGET, Br. J. Derm. *75*, 105 (1963).

[7] A. F. GARACIA and GRINSTEIN, Biochem. Pharmac. *16*, 1967 (1967).

Recently, WADA et al.[8] demonstrated that some porphyria-inducing agents may block the pathway from heme formation to heme utilization for hemoprotein synthesis. Therefore, it is well recognized that there is a direct correlation between occurrence of experimental porphyria and changes in hemoprotein levels.

On the other hand, GREENGARD et al.[9–11] reported that the induction of TP by the substrate is dependent upon the degree of saturation of apo-TP with respect to a co-factor, protohemin IX.

As indicated in a preceding paper and shown in Figure 1 in this paper, TP activity of non-induced rats was activated by PHZ treatment. This phenomenon was presumably due to the mechanism similar to that reported by others[12,13], that is, stimulation of porphyrin metabolism in porphyric animals appears to raise the level of apo-TP. As demonstrated in the present study, accumulated free porphyrins in urine seems to disturb the tryptophan-mediated TP induction because inhibition of TP by added protoporphyrin was also reported in vitro experiments[14,15].

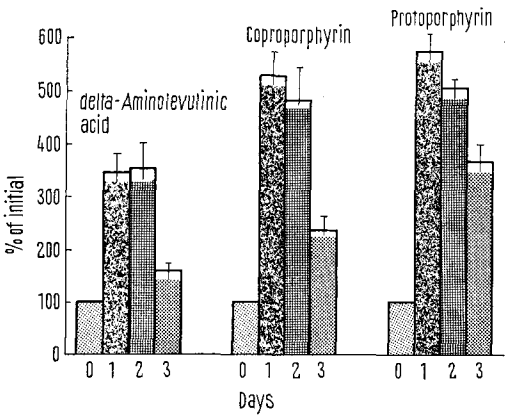

Fig. 2. Increases in urinary delta-aminolevulinic acid, coproporphyrin and protoporphyrin levels in phenylhydrazine-treated rats. Animals were administered 40 mg/kg of phenylhydrazine i.p. Urines of 2 rats were mixed and determined for 3 components during 3 successive days after treatment.

In conclusion, the results obtained here suggested that induction process of TP by the substrate, namely, conjugation of apo-TP with respect to protohemin IX seemed to be, in part, prevented competitively by an increased free porphyrins in PHZ-treated rats.

Zusammenfassung. Nachweis, dass Phenylhydrazin die von Tryptophan, nicht aber von Kortison hervorgerufene Induktion der Tryptophanpyrrolase in der Rattenleber hemmt. Die Hemmung der Enzyminduktion durch Phenylhydrazin dürfte auf Anomalien des Porphyrinstoffwechsels beruhen.

T. SATOH and K. MOROI

Department of Pharmacology and Toxicology, Institute of Food Microbiology, Chiba University, Chiba (Japan), 14 July 1969.

[8] O. WADA, Y. YANO, G. URATA and K. NAKAO, Biochem. Pharmac. *17*, 595 (1968).
[9] O. GREENGARD and P. FEIGELSON, Nature *190*, 446 (1961).
[10] O. GREENGARD and P. FEIGELSON, J. biol. Chem. *236*, 158 (1961).
[11] O. GREENGARD and G. ACS, Biochim. biophys. Acta *61*, 652 (1962).
[12] P. FEIGELSON and O. GREENGARD, Biochim. biophys. Acta *52*, 509 (1961).
[13] P. FEIGELSON, M. FEIGELSON and O. GREENGARD, *Recent Progress in Hormone Research* (Academic Press Inc., New York 1961), p. 491.
[14] O. GREENGARD and P. FEIGELSON, J. biol. Chem. *237*, 1903 (1962).
[15] P. FEIGELSON and O. GREENGARD, J. biol. Chem. *237*, 1908 (1962).

Multiple Histidine Degrading Enzymes in *Proteus vulgaris*

Much work has been carried out on the pathways of biological degradation of histidine. Histidase (E.C.4.3.1.3), transaminases, decarboxylases (E.C.4.1.1.22) and oxidases (E.C.1.4.3.2) concerned with the L-amino acid are known to exist side-by-side in the same tissue in higher animals[1–6]. Similar pathways are found in bacteria[7–15]. The collective presence in higher animals of these various pathways is of prime importance to provide requisite intermediary metabolites and pharmacologically active substances[16] while transamination and oxidative deamination have different advantages and disadvantages[17,18]. However, there have been few reports of multiple mechanisms existing concurrently in microorganisms while the possible presence of 2 histidine transaminases such as found in *Neurospora*[19–21] is due to one of them being primarily involved in the biosynthesis of the amino-acid rather than in its breakdown.

Recent work has established the presence of an L-histidine transaminase in *Escherichia coli*[7,8]. The enzyme was found to be repressed when the cells were cultivated under conditions of adequate 'available' nitrogen[9]. A histidine decarboxylase is however produced under other quite different conditions in *E. coli* (and *Proteus*)[15,22]. In view of this interesting ability of *E. coli* to produce alternative (though not co-existent) pathways of histidine degradation it was felt to be of interest to investigate whether a micro-organism known to oxidize its histidine using an L-amino acid oxidase produces a transaminase when cultured as were *E. coli*[9]. Since the L-amino acid oxidases of *Cl. sporogenes* and *Cl. saccharobutyricum* do not attack histidine[23], it was decided to use *P. vulgaris*[10]. Recent work[24] has confirmed the conversion of histidine to imidazolepyruvic acid by *P. vulgaris* in vivo, but the enzymes involved were not investigated in this species.

The method of cultivation of cells of *P. vulgaris* wild strain A 232 (collection of the Pasteur Institute, Paris), the preparation of a crude enzyme extract and the spectrophotometric assay have been described[9]. The assay (adapted from [5]) utilizes the absorption at 293 nm of the complex imidazolepyruvate:borate formed in the presence of arsenate. Exhaustive overnight dialysis of the enzyme extract against 500 vol. of 20 m*M* sodium phosphate buffer (pH 7.2) eliminates endogenous amino-acceptor substances[7].