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].

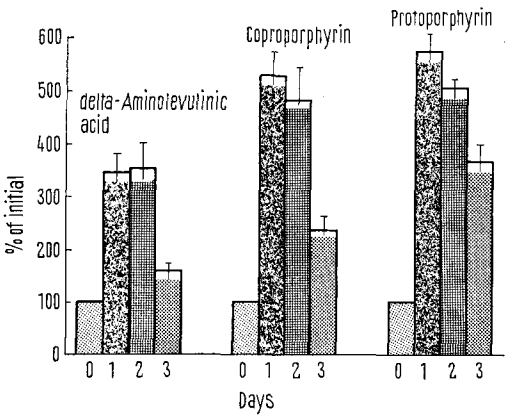

Fig. 2. Increases in urinary delta-aminolevulinic acid, coproporphyrin and protoporphyrin levels in phenylhydrazine-treated rats. Animals were administered 40 mg/kg of phenylhydrazine i.p. Urines of 2 rats were mixed and determined for 3 components during 3 successive days after treatment.

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.

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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.

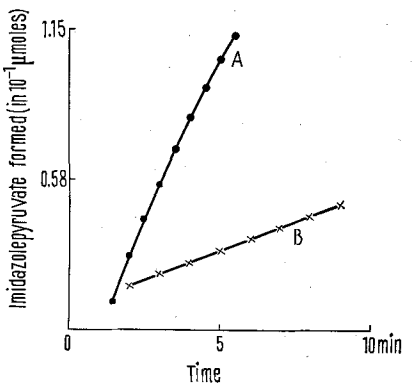
The method of cultivation of cells of *P. vulgaris* wild strain A 232 (collection of the Pasteur Institute, Paris), the preparation of a crude enzyme extract and the spectrophotometric assay have been described[9]. The assay (adapted from [5]) utilizes the absorption at 293 nm of the complex imidazolepyruvate:borate 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].

The assay was carried out both in the absence (Curve B) and in the presence (Curve A) of α-ketoglutarate and pyridoxal phosphate. The Figure shows that while imidazolepyruvic acid was formed in both cases, the L-amino acid oxidase (Curve B) had a much lower activity than the combined oxidase *plus* transaminase (Curve A). The activities observed were those at the standard assay conditions of pH 8.0. Further assays were carried out in the absence of amino-acceptor and cofactor but incorporating 23 m*M* EDTA to inhibit any histidase present[5]. The progress of these reactions corresponded with Curve B, which showed the absence of histidase in the extracts.

Further incubations of the dialysed crude extract at pH 7.2 were carried out as before[7,8] and the deproteinized supernatants chromatographed on paper (descending run, solvent – upper phase, *n*-butanol:acetic acid:water, 50:12:50) or electrophoresed on paper at 6000 V for 30 min (buffer – formic acid:acetic acid, pH 1.9). Imidazoles were revealed by spraying with diazotized *p*-chloroaniline[25]. These experiments showed that a single imidazole product formed which corresponded to imidazolepyruvate.

The above results demonstrate the presence of a histidine transaminase of unknown specificity in *Proteus vulgaris*. This enzyme was found to be much more active in the crude extract than the L-amino acid oxidase (i.e. 1.35×10^{-2} μmoles histidine converted/min/mg crude protein and 0.27×10^{-2} μmoles histidine converted/min/mg crude protein respectively). The apparently low specific activity of the amino acid oxidase could also have been caused by the pH of the assay system being sub-optimal, the enzyme production having been repressed by the conditions of culture or by substrate inhibition as is the case with mammalian L-amino acid oxidase[26] (although the bacterial and mammalian enzymes are dissimilar in other properties[23]). Histidine decarboxylase[15] is not produced under these conditions of culture.

Earlier work demonstrated that the histidine transaminase of *E. coli* is produced under conditions of 'nitrogen starvation'[9], presumably to increase the ability of the organism to utilize and transfer nitrogen from available sources. It will be of interest to see if the transamination of histidine to give glutamic acid by *P. vulgaris* corresponds to that of *E. coli* and whether the production of dicarboxylic keto acids[8,27] and pyridoxal phosphate, the cofactor of amino acid transporting and degrading enzymes rises in conditions of low nitrogen.



Formation of imidazolepyruvate from histidine by crude dialyzed extracts of *Proteus vulgaris*. Spectrophotometric assay at 293 nm of product complexed with borate[9]. Protein concentration 0.46 mg/ml. ●—● Curve A, 7.14×10^{-2} *M* histidine, 7.4×10^{-2} *M* α-ketoglutarate and 10 μg/ml pyridoxal phosphate added. Product formation 1.62×10^{-2} μmoles/min/mg protein. X—X Curve B, (1) 7.14×10^{-2} *M* histidine only added, (2) 7.14×10^{-2} *M* histidine and 23 m*M* EDTA added. Product formation in either case 0.27×10^{-2} μmoles/min/mg protein.

Résumé. Bien que l'histidine décarboxylase soit absente chez *Proteus vulgaris* cultivé dans les conditions décrites, une histidine transaminase se montre en même temps que l'oxydase L-amino acide déjà connue. La transamination est bien plus rapide que l'action L-amino oxydasique.

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