*Int. J. Biochem.* Vol. 12. pp. 947 to 953

0020-711X/80/1101-0947$02.00/0

# PORPHYRIN BIOSYNTHESIS AND ENZYMIC STUDIES IN ERYTHROCYTES FROM NORMALS AND PORPHYRIC HUMANS

VICTORIA E. PARERA, ANA MARÍA STELLA, EVA A. WIDER DE XIFRA, HAYDÉE FUKUDA and ALCIRA M. DEL C. BATLLE
Centro de Investigaciones sobre Porfirinas y Porfirias, CIPYP, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires y Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Ciudad Universitaria-Pabellón II 4° Piso, 1428 Buenos Aires, Argentina

**Abstract**—1. Studies on porphyrin biosynthesis from exogenus ALA, at various time intervals as well as direct enzyme measurements (aminolevulinic acid dehydratase (ALA-D); porphobilinogenase (PBG ase) and deaminase were carried out in hemolysates of human erythrocytes from healthy controls and patients with lead poisoning (Pb), acute intermittent porphyria (AIP), porphyria cutanea tarda (PCT), erythropoietic protoporphyria (EPP), variegate porphyria (VP) and congenital erythropoietic porphyria (CEP).

2. Inhibited ALA-D in Pb, reduced PBGase and deaminase in AIP, lower uroporphyrinogen decarboxylase in PCT, and diminished isomerase in CEP, were confirmed. In addition, ALA-D was found, reduced in AIP, unchanged in PCT and increased in EPP, VP and CEP. PBGase and deaminase were, on the other hand, increased in Pb and PCT, unchanged in VP and diminished in EPP and CEP.

3. Total porphyrin biosynthesis is a function of time; compared to normals, is lower in CEP and AIP, but higher in PCT.

4. The porphyrin profile changes along the time; uroporphyrin increases at longer intervals while that of coproporphyrin concomitantly diminished. A significance enhancement of octacarboxylic porphyrins was observed during the entire duration of the incubation in PCT hemolysates. In CEP the main porphyrin was always uroporphyrin I.

5. Studies on both total porphyrins formed and their distribution were performed in hemolysates from cases of non-hereditary and hereditary PCT and AIP, before and after therapy.

## INTRODUCTION

The porphyrias, either inherited or acquired are a group of diseases characterised by a combination of enhanced synthesis, accumulation and excretion of porphyrins and/or their precursors, δ-aminolevulinic acid (ALA) and porphobilinogen (PBG), due to an enzymic disorder in porphyrin metabolism.

Besides their distinctive clinical syndromes, their unique and typical biochemical patterns of excretion are very useful tools for distinguishing each porphyria. However, we are aware that the sole study of the excretion profiles in urine and feces cannot always provide enough information to establish a differential diagnostic nor on the site of abnormal synthesis, that is why it is often necessary and advisable to determine the specific enzyme deficiency.

It has been already observed by others and in our laboratory (Doss, 1973; Schermuly & Doss, 1976; Viljoen *et al.*, 1976; Miyagi *et al.*, 1976; Batlle *et al.*, 1979a,b), that information about the underlying enzymic defect and inheritance in many porphyrias can also be obtained by carrying out *in vitro* studies on porphyrin biosynthesis from ALA and PBG by erythrocytes, under different experimental conditions as well as by direct enzyme measurements.

We will only report here, results on porphyrin biosynthesis from exogenous ALA at various time intervals, by human erythrocytes hemolysates from normals and patients with lead poisoning (Pb), acute intermittent porphyria (AIP), porphyria cutanea tarda (PCT), erythropoietic protoporphyria (EPP), variegate porphyria (VP) and congenital erythropoietic porphyria (CEP). The activities of δ-aminolevulinic acid dehydratase (ALA-D), porphobilinogenase (PBGase) and deaminase were also determined in the same hemolysates.

## MATERIALS AND METHODS

Blood samples were obtained and hemolysates prepared following Batlle *et al.* (1978).

Incubation conditions were those reported by Batlle *et al.* (1979b). Incubation time varied between 2 and 8 hr. Fractionation, identification and quantitative spectrophotometric determination of the porphyrinogens formed as well as other methods and materials not specified here were those already described by Llambias & Batlle (1971a) and Batlle *et al.* (1979a). Total rate of biosynthesis is expressed as nmol of porphyrin per ml of RBC. ALA-D was assayed by the method of Batlle *et al.* (1967). PBGase and deaminase were measured as described by Batlle *et al.* (1978).

Enzyme units; one unit of enzyme activity is defined as the amount of enzyme which catalyses the formation of 1 nmol of product in 60 min under the standard incubation conditions, and enzymic activities are expressed as units per ml of RBC.

## RESULTS AND DISCUSSION

### *Enzyme determinations*

Findings obtained are presented in Fig. 1. In lead poisoning as it is very well known ALA-D is signifi-

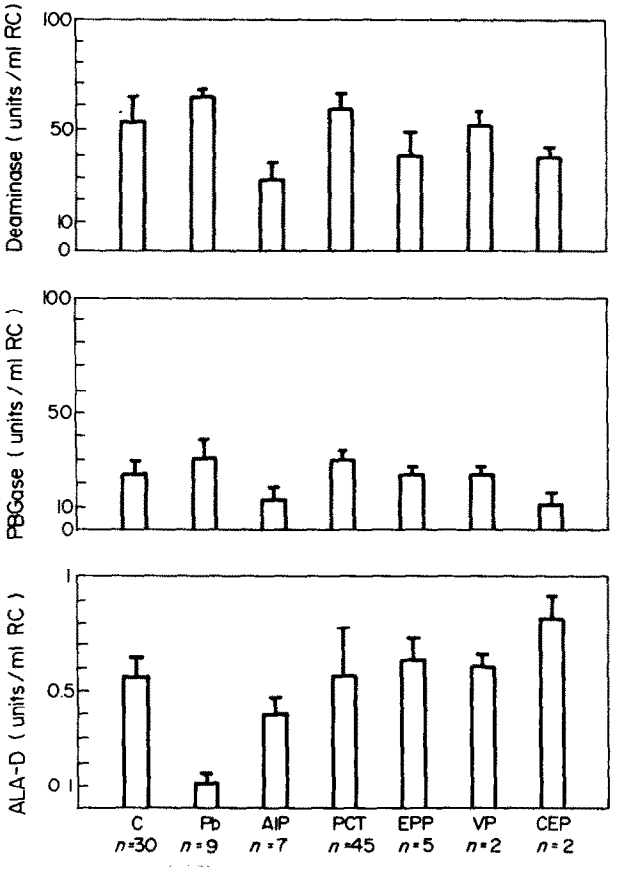


Fig. 1. Enzyme determinations. Every measurement was carried out by duplicates and in many patients repeated periodically. n = number of cases.

cantly inhibited, but it was consistently found here, that both PBGase and deaminase activities were 15–20% increased. These results are not easy to explain because it has been demonstrated that *in vitro*, μmolar to mmolar concentrations of lead, depending on the tissue, inhibited PBG consumption and porphyrin formation by these enzymes (Llambías & Batlle, 1971b; Piper & Tephly, 1974; Tephly, 1979; Sancovich *et al.*, 1976). However it is interesting to add, that PBG urinary levels in these patients were normal; probably, accumulation of lead in tissues was not high enough to *in vivo* inhibit conversion of PBG to uroporphyrinogen, leading to its accumulation and its finally increased excretion in urine. It therefore appears that depending on whether or not PBG is excreted in excess in urine, we could expect enzymic activity levels of erythrocytes PBGase and deaminase be lower or higher than normals.

It was found that in AIP, not only PBGase and deaminase were reduced to half but also ALA-D activity was 25–30% diminished. In PCT either inherited or acquired, as an average ALA-D was not modified, however values obtained were within a great range of variability, while PBGase and deaminase were slightly augmented; in some cases where ALA-S was determined, this activity was 25–50% increased.

Curiously in EPP, VP and CEP, ALA-D was again about 30, 20 and 50% respectively, above normal; ALA-D has also been found to be enhanced in blood and liver of a bull with CEP (Batlle *et al.*, 1979a), moreover, we and others have observed that in both patients and animals with CEP, ALA-S was 2–4 times increased in blood and bone marrow (Moore *et al.*, 1978; Batlle *et al.*, 1979a); therefore it could be, that in the last four types of porphyria the observed enhancement in ALA-D activity is in fact a reflex of an excess of substrate. PBGase was unchanged in EPP and VP but around 60% reduced in CEP, while deaminase was 20% lower in EPP and CEP. These findings would suggest that at least in these two CEP patients a decreased activity of isomerase might be the primary enzymic defect, in agreement with the hypothesis of Levin (1968). However it has been demonstrated that in some CEP human and animal cases, both decreased activity of isomerase and increased activity of deaminase are co-existent (Moore *et al.*, 1978; Batlle *et al.*, 1979a), supporting the view that CEP is phenotypically homogeneous in most respects but genetically heterogeneous, that is, that the inborn error in porphyrin biosynthesis may vary among different types of CEP (Hofstad *et al.*, 1973). In general we would say that meaningful conclusions can only be drawn by studying individual cases.

*Biosynthetic activity*

*Total porphyrin biosynthesis.* Although porphyrias might be primarily liver or bone marrow disturbances, studies on porphyrin biosynthesis from the precursors. ALA and PBG by red cell hemolysates may

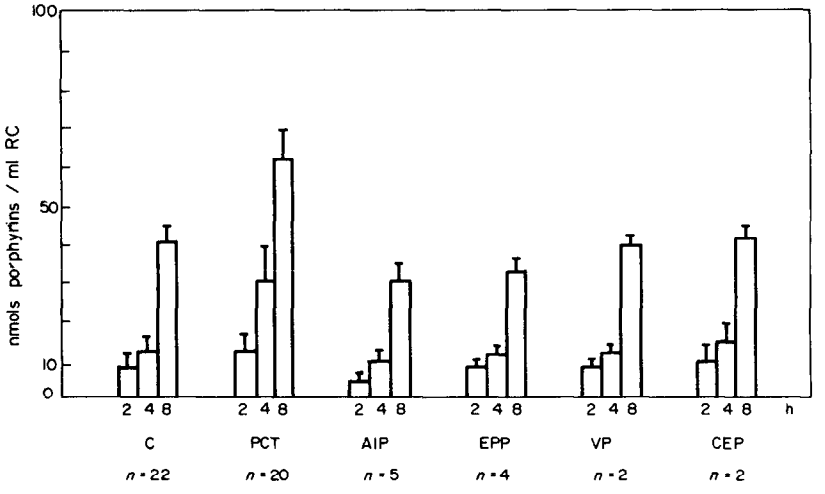


Fig. 2. Total porphyrin biosynthesis by hemolysates from healthy control (C) and different porphyric patients, after 2, 4 and 8 hr of incubation. Every experiment was run by duplicates.

be of significance in the interpretation of porphyria excretion patterns and advance information about the specific enzymic defect and inheritance. They have also the advantage that synthesis cannot go beyond protoporphyrin, thus making easier the interpretation of porphyrin patterns.

As we are here reporting results obtained using ALA as substrate, lead poisoning cases will be excluded from now on.

In Fig. 2, total porphyrin formed by hemolysates from healthy volunteers and different porphyrics at 2, 4 and 8 hr is illustrated. It must be noted that longer incubations, up to 24 hr were also performed, however the longer the time the greater the proportion of isomers I found; therefore it is not possible to make valid inferences from those experiments.

As it is well known, in general, total porphyrin biosynthesis is a function of time (Doss, 1976; Batlle *et al.*, 1979a,b). In PCT, porphyrin formation is significantly increased as compared to controls. So far, these findings are in agreement with an augmented urinary excretion of porphyrins and would suggest that the activity of one or more of the enzymes after ALA-S might be elevated. It was found, in fact, that PBGase and deaminase were slightly higher than normal (Fig. 1) and Doss (1976) postulated that the activity of deaminase can be increased in PCT. However, they do not explain, yet, the typical constellation of porphyrins excreted in PCT; an analysis of the distribution of porphyrins is therefore compulsory.

As expected, at shorter intervals, porphyrin synthesis in AIP was reduced to 45–50% (Doss, 1973; Batlle *et al.*, 1979b), this might be due to the known enzymic defect of deaminase, but we should also take into account the diminished activity of ALA-D here found; which all together well agree with the abnormal excessive quantities of PBG and ALA excreted in AIP urine, and can be of diagnostic usefulness.

On the other hand, no significant differences are apparent between total porphyrins formed in EPP, VP and CEP and in controls, so the determination of their porphyrin biosynthesis pattern is necessary to get information as to the type of porphyrins and isomers formed.

*Total porphyrin biosynthesis in PCT and AIP.* We considered it interesting to determine if there were

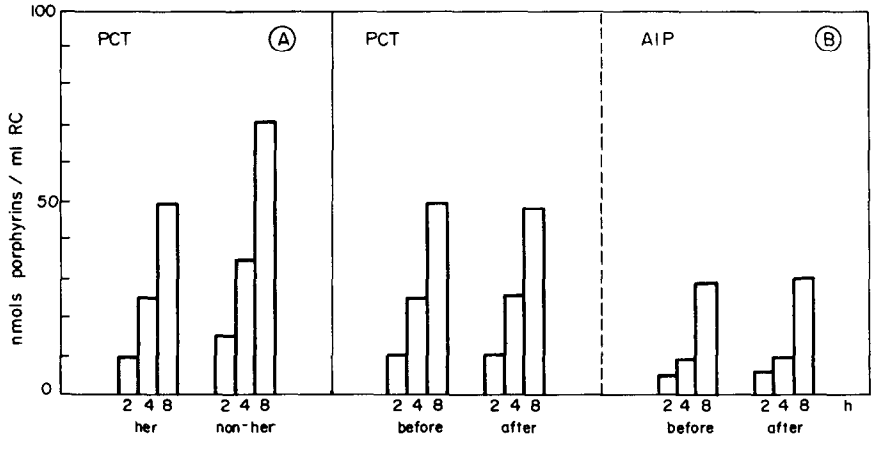


Fig. 3. Total porphyrin biosynthesis, after 2, 4 and 8 hr of incubation of hemolysates from, Part A: hereditary (her) and non-hereditary (non-h.) PCT patients; Part B: PCT and AIP patients before and after therapy.

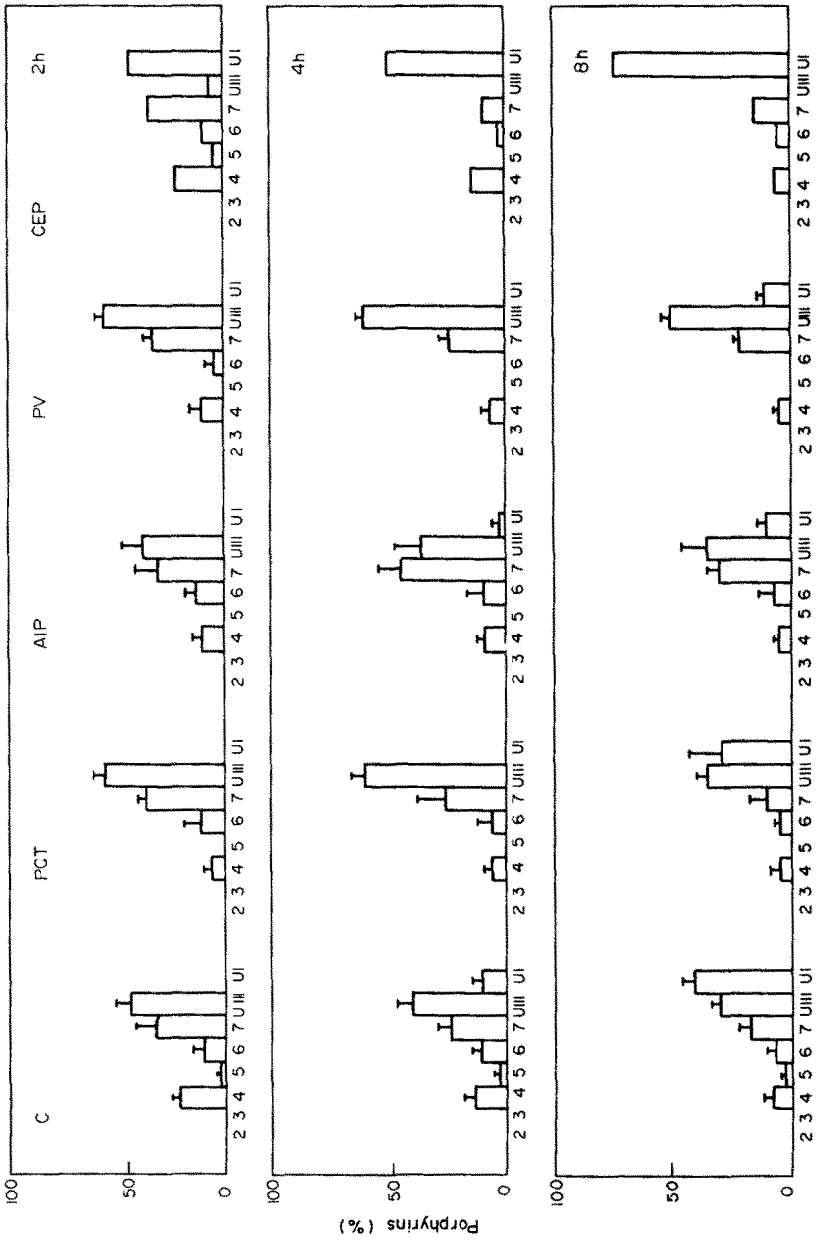


Fig. 4. Porphyrin biosynthetic pattern corresponding to porphyrins formed by incubating hemolysates from the healthy controls (C) and different porphyric patients illustrated in Fig. 2.

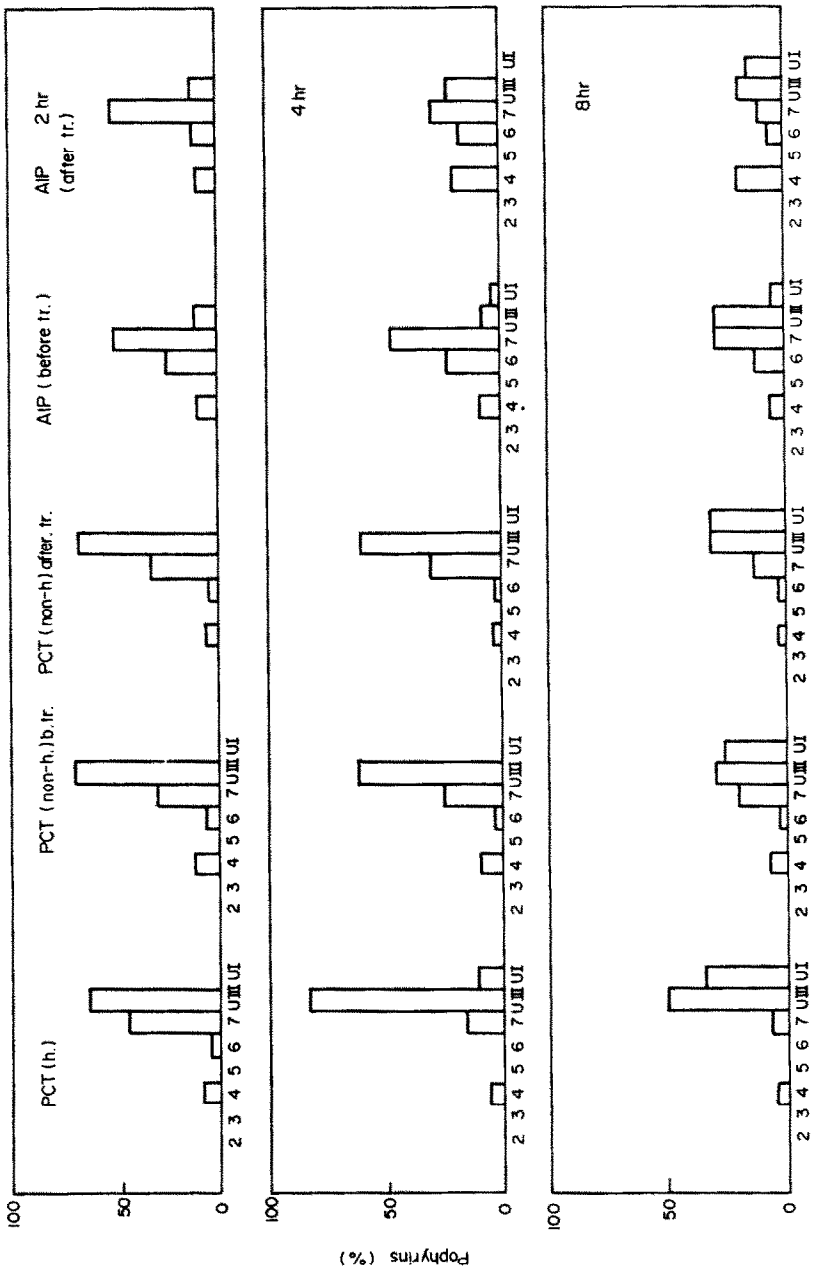


Fig. 5. Porphyrin biosynthetic pattern in cases of hereditary (h.) and non-hereditary (non-h.) PCT and AIP, before and after treatment (tr.)

any changes in the porphyrin biosynthesis by hemolisates from hereditary PCT and AIP patients, before and after therapy. As shown in Fig. 3, Part B, the answer to the first question was clearly negative. On the other hand, (Part A), although in both hereditary and non-hereditary PCT synthesis was 1.3 to 2 times as high as in controls. There was consistently a clear cut difference between one and the other, being greater in the latter, which was correlated with a higher increase in PBGase and deaminase activities, confirming previous results (Batlle *et al.*, 1979b).

*Distribution of biosynthetic porphyrins.* The pattern corresponding to porphyrins synthesized by hemolysates of controls and porphyrics from ALA, after 2, 4 and 8 hr of incubations is shown in Fig. 4. In general the proportion of highly carboxylated porphyrins, mainly Uro, increases with time, while the percentage of less carboxylated, as Copro, concomitantly decreases.

It has been confirmed that in PCT porphyrin distribution is different from normal and quite distinctive, after 2 hr there is an increased synthesis of Uro and heptacarboxylic porphyrins as well as reduced percentages of hexa and Coproporphyrins, although their absolute concentrations were within normal values. These results are clearly indicative of a defect in the activity of Uroporphyrinogen decarboxylase, which was observed in both symptomatic PCT patients and relatives with latent PCT (Doss, 1976; Batlle *et al.*, 1979b) and can also be of diagnostic significance.

It is interesting that, at shorter time intervals the distribution of porphyrins in AIP is quite similar in patients and controls (Doss, 1973), except that the percentage of Copro is lower, and that of Uro III slightly higher; we must remember that the absolute amount of total porphyrins was 45–50% lower, which would be compatible with the demonstrated defect in deaminase and also ALA-D activity.

A different pattern, quite similar to that seen in PCT, was found for the distribution of porphyrin in VP. In these instances we do know that the qualitative analysis of the porphyrins excreted in both urine and feces is of great importance for a differential diagnostic. As we have observed that in VP, ALA-D, PBGase and deaminase seem not to be modified, it could be interesting to measure the levels of uroporphyrinogen decarboxylase, as the proportion of highly carboxylated porphyrins is so high.

In CEP, the general picture is not much different from controls, but uroporphyrinogen I is the dominant isomer. Decarboxylation is apparently not affected, but the lower proportion of Uro III, seems to reflect a defect at the level of isomerase, which is in agreement with both the typical constellation of porphyrins excreted in CEP, and enzymic determinations. However as is has already been discussed, CEP, a rare disease, might be genetically expressed in different types. Nevertheless, these kind of studies are useful for distinguishing the nature of the inborn error.

*Porphyrin biosynthetic patterns in cases of hereditary and non-hereditary PCT and AIP, before and after therapy.* It was shown (Fig. 2), that there were differences between the total amount of porphyrins formed by hemolysates from patients with non-hereditary and hereditary PCT. We wanted to know if those differences were only quantitative or if there was also any qualitative change. In addition, the distribution of biosynthetic porphyrins was further studied in PCT and AIP patients before and after therapy.

As we can see in Fig. 5, the pattern produced by hereditary PCT is somehow different from that found in non-hereditary PCT. After 4 and 8 hr incubation the percentage of 8-carboxylic porphyrins in the former is greater than in the latter, as if the first decarboxylation step were more inhibited here, although after 2 hr, the effect was apparently opposite.

As far as treatment is concerned, it did not modify the picture in PCT; however, in one AIP patient, after folic acid therapy (Wider de Xifra *et al.*, 1979), the initial pattern, which was anyhow unusual before therapy, showed a tendency to normal, but only after 4 hr incubation; more recently, these findings were confirmed with another AIP patient subjected to the same treatment. Nevertheless, we have not enough experimental data to claim that the analysis of the biosynthetic pattern can be useful in following the course of the therapy in AIP, and further studies are indeed necessary to support or not these results. Moreover as stated above, in many cases it is not always possible to arrive at valid conclusions from mean values, and in these instances it is highly advisable to carry out complete individual studies.

*Acknowledgements*—This work was supported by grants from the CONICET, the University of Buenos Aires and the SECYT. A. M. del C. Batlle, E. A. Wider de Xifra and A. M. Stella hold posts as Scientific Researchers in the CONICET. This work forms part of the thesis of V. Parera to be submitted to the University of Buenos Aires for her Ph.D. degree. H. Fukuda thanks the CONICET for the award of a Research Fellowship. Blood samples were obtained from healthy volunteers, different porphyric patients and their relatives who were referred by Professor P. H. Magnin and Dr S. Malamud, Clinical follow up and responsability for treatment remained with the referring physician. We are very grateful to Dr A. A. Juknat de Geralnik and Dr M. V. Rossetti for their valuable help in obtaining venous blood and also to Miss H. Gasparoli, Mrs I. Jaievsky and Mr D. Sachetti for their skillful technical assistance.

## REFERENCES

BATLLE A. M. DEL C., FERRAMOLA A. M. & GRINSTEIN M. (1967) Purification and general properties of ALA-D from cow liver. *Biochem. J.* **104,** 244–249.

BATLLE A. M. DEL C., WIDER DE XIFRA E. A. & STELLA A. M. (1978) A simple method for measuring erythrocyte porphobilinogenase, and its use in the diagnosis of acute intermittent porphyria. *Int. J. Biochem.* **9,** 871–875.

BATLLE A. M. DEL C., WIDER DE XIFRA E. A., STELLA A. M., BUSTOS N. & WITH T. K. (1979a) Studies on porphyrin biosynthesis and the enzymes involved in bovine congenital erythropoietic porphyria. *Clin. Sci.* **57,** 63–70.

BATLLE A. M. DEL C., WIDER DE XIFRA E. A., STELLA A. M., BUSTOS N., VAZQUEZ E. & MAGNIN P. H. (1979b) Porphyrin biosynthesis in normals and porphyrics from precursors. *Revta argent. Derm.-Sif.* **60,** 102–107.

DOSS M. (1973) Metabolism of δ-aminolevulinic acid and porphobilinogen in human erythrocytes in acute intermittent porphyria. *Enzyme* **16,** 343–353.

HOFSTAD D., SEIP M. & ERIKSEN L. (1973) Congenital erythropoietic porphyria with a hitherto undescribed porphyrin pattern. *Acta pediat. scand.* **26,** 380–384.

LEVIN E. (1968) Uropophyrinogen III cosynthetase in bovine erythropoietic porphyria. *Science* **161**, 907–908.

LLAMBIAS E. B. C. & BATLLE A. M. DEL C. (1971a) Studies on the porphobilinogen deaminase-uroporphyrinogen cosynthetase system of cultured soybean cells. *Biochem. J.* **107**, 327–340.

LLAMBIAS E. B. C. & BATLLE A. M. DEL C. (1971b) Porphyrin biosynthesis VII-Avian erythrocyte porphobilinogen deaminase-uroporphyrinogen III cosynthetase, its purification, properties and the separation of its components. *Biochim. Biophys. Acta* **227**, 180–191.

MIYAGI K., PETRYKA Z. J., BOSSENMAIER I., CARDINAL R. & WATSON C. J. (1976) The activities of uroporphyrinogen synthetase and cosynthetase in congenital erythropoietic porphyria (CEP). *Am. J. Hemat.* **1**, 3–21.

MOORE M., THOMPSON G. G., GOLDBERG A., IPPEN H., SEUBERT A. & SEUBERT S. (1978) The biosynthesis of haem in congenital erythropoietic porphyria. *Int. J. Biochem.* **9**, 933–938.

PIPER W. N. & TEPHLY T. R. (1974) Differential inhibition of erythrocyte and hepatic uroporphyrinogen synthetase activity by lead. *Life Sci.* **14**, 873–876.

SANCOVICH H. A., FERRAMOLA A. M., BATLLE A. M. DEL C., KEVILEVICH A. & GRINSTEIN M. (1976) Studies on cow liver porphobilinogen deaminase. *Acta physiol. latinoam.* **26**, 379–385.

SCHERMULY E. & DOSS M. (1976) Porphyrin biosynthesis from ALA and PBG by human erythrocytes in porphyrin disorders. Kinetic studies of the isomer series I and III. *Ann. clin. Res.* **8**, suppl. 17, 92–102.

TEPHLY T. R. (1979) Identification of a small molecular weight substance that protects Uroporphyrinogen I Synthetase from the inhibition by lead. In *Porphyrins in Human Diseases—Report of Discussions* (Edited by DOSS M. & NAWROCKI P.) pp. 128–134. Falk Freiburg.

VILJOEN J. D., BECKER D. & KRAMER S. (1976) A comparative study of porphyrin synthesis by whole blood and haemolysates from different mammalian species. *Comp. Biochem. Physiol.* **55B**, 151–155.

WIDER DE XIFRA E. A., BATLLE A. M. DEL C., STELLA A. M. & MALAMUD S. (1979) Acute Intermittent Porphyria—Another approach to therapy. *Int. J. Biochem.* **12**, 819–822.