Dr. Bhaskar Menon Ambady Oration 1980

PORPHYRIN IN HEALTH AND DISEASE

F. HANDA *

The purposes of my presentation are two-fold: first, to memorialize Dr. Bhaskar Menon Ambady; second, to present to you an over-view of my research work done on porphyrin in health and disease during the last decade.

It is a sad and difficult duty to give a fitting recognition of a dermatologist and investigator who led such a fruitful life as did Dr. Ambady. Though an untiring worker in his chosen field of Dermatology, he found time to be a well-rounded man. He was president of the Weight Lifters Association, president of Wrestling Association and vice-president of Foot-Ball Association.

To turn to his academic career, which was long and productive, he received his M.B.B.S. degree from the Madras Medical College. He had training in various international institutions and was pioneer in setting up the department of dermatology and venereology at Medical College, Trivandrum. He was the founder member and president of Indian Association of Dermatologists, Venereologists and Leprologists under whose aegis we are meeting today. Dr. Ambady disliked pomp and show, was cosmopolitan in his outlook and a real gem of a person.

Sri Vivekananda has said, "This life is short, the vanities of the world are transient, but they alone live who live for others, the rest are more dead than alive". Dr. Ambady believed in this philosophy of life. In attempting to summarize his fruitful life, I can do no better than paraphrase what has already been said about him on earlier occasions.

I have chosen the subject of porphyrin in health and disease to commemorate his undying interest in dermatology.

Porphyrins in Health

The porphyrins are purple-red pigments (Greek, porphuros-purple) which are contained within every cell of the human body and are responsible for the red colour of the blood by their presence in haemoglobin as an iron-protoporphyrin complex or haem, a substance which also forms a part of many intracellular enzymes. So important are the porphyrins for the proper functioning of the most lowly as well as the most developed forms of life in both animal and plant kingdoms that porphyrin can be truly labelled as the "Colour of Life". This colour is reflected in all living things of nature, namely, red colour of azaleas

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* Professor and Head, Department of Skin and V.D., Government Medical College, Patiala (Punjab).

in blossom, red colour of star-fish, porphyrin red feathers of flemingo and the fluorescent human red-blood corpuscles from a case of Gunther's disease. How beautiful indeed do these look !

Biological functions of porphyrins are so important and varied indeed that one wonders whether the evolution of life began with the simultaneous evolution of porphyrin pigments itself.

According to Adler[1], biological roles of porphyrins are diverse, namely, photosynthesis, photodynamic action, respiration, in enzymes, electron transport, gas transport, storage and exchange, pigmentation in shells, feathers, urine, etc; porphyrias, haemoglobinopathies, cancer; food technology : beer, cheese, 'colour'; petroleum technology : catalysts, 'spills'; evolution and cosmology; history, archeology and anthropology.

Porphyrin was first discovered by Thudichum[2] in 1867. In 1871, Hoppe-Seyler[3] isolated it from blood. The chemistry of porphyrin has been worked out by Hans Fischer and Orth[4], Lemberg[5] and MacDonald[6].

Porphyrins are cyclic compounds formed by the linkage of four pyrrole rings, connected by methene bridges (=CH).

Structure of porphobilinogen is given in Fig. 1.

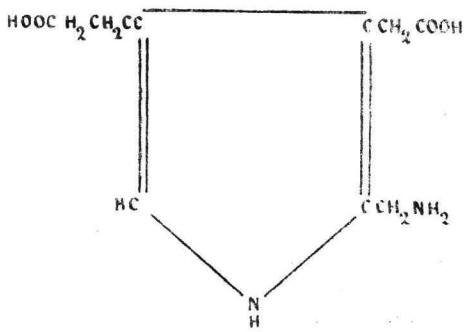

Fig. 1 Structure of Porphobilinogen.

Porphyrins are mainly synthesised in bone-marrow and liver. Stages of porphyrin synthesis are given in Fig. 2.

Normally the body is most economical with the porphyrin it forms and only traces of them are excreted in the urine and stools. In porphyrias however, there is vastly increased formation and excretion of porphyrins or some of its precursors.

Determination of normal porphyrin levels in blood, urine and stools

It is important to determine the normal values of porphyrin in Indians because these values vary in different races and in individuals of the same race. Most of the studies have been conducted in European Whites, few in African coloured and none in Indians. It will not be correct to apply the values determined in other races while assessing cases of disordered porphyrin metabolism in our people. Hence, there is need to study normal porphyrin levels in Indians, and to collect baseline data.

Quantitative determination of porphyrin was done with Rimington's[7] technique. Its extraction and spectrophotometric determination were done after standardising the procedure. Porphyrins being photolabile, the extraction was done in dark room after taking all the necessary precautions.

Normal quantitative values of porphyrin and its precursors as reported in white races compared with values in Punjabis (Indians) are given below :—

A. Normal Blood Porphyrin in Punjabis (Indians)

The erythrocyte blood porphyrin in twenty-five normal Punjabis were determined by Handa and Adarsh[9] and this study was extended to another 100 normal Punjabis by Adarsh, Handa and Sidhu[10]. Results are tabulated in

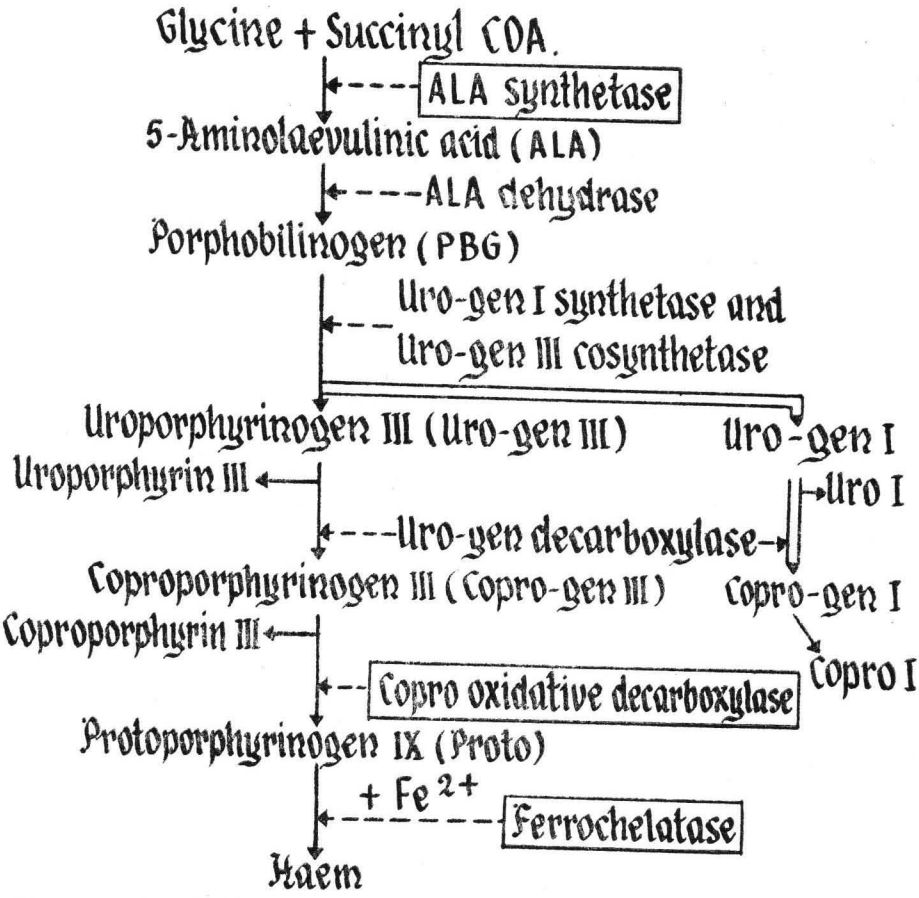

Fig. 2 - Simplified scheme of main features of the porphyrin biosynthetic pathway. Enzymes shown in boxes are intra-mitochondrial, others lie in cell sap. Pathway indicated in double line with series I isomers normally insignificant, greatly increased in Gunther's Disease and PCT.

table 1. The erythrocyte coproporphyrin level ranged from 0–4.99 ug/100 ml R.B.C.s with a mean value of 0.22 (±0.667). The erythrocyte protoporphyrin level ranged from 2.26 – 83.9 ug/100 ml R.B.C.s with a mean value of 25.03 (±13.818).

The effect of age, sex and body weight, diet and haemoglobin were also studied on coproporphyrin as well as protoporphyrin of erythrocytes. While sex had no effect on the levels of porphyrin, erythrocyte protoporphyrin levels in the age group 0–14 years were significantly higher than in the group 15 – 39 and forty years above. Body weight had no significant effect. Haemoglobin showed significant relationship to protoporphyrin levels and not to those of coproporphyrin.

Cord blood porphyrins in thirty new born babies were determined by

TABLE 1

Normal quantitative values of porphyrins in urine, stools and R.B.Cs of Punjabis (Indians) compared with those of whites (given in brackets) (Goldberg, 1966)[8]

Urinary	
Copro :	12.93 - 117.85 (0-280) ug/24 h
Uro :	0-22.71 (0-40) ug/24 h
PBG :	Under study (0-0.5) mg/24 h
ALA :	Under study (0-5.0) mg/24 h
Faecal	
Copro :	0.29 - 3.52 (0-50) ug/g dry wt
Proto :	1.05 - 14.91 (0-115) ug/g dry wt
'X' :	Under study (0-20) ug/g dry wt
R.B.C 's	
Copro :	0-4.99 (0-4) ug/100 ml cells
Proto :	2.26-83.9 (0-30) ug/100 ml G

Copro—Coproporphyrins
PBG —Porphobilinogen
Proto —Protoporphyrin
Uro —Uroporphyrins
ALA —d-Aminolevulinic acid

F. Handa and S. C. Sharma[11], and this study was extended to another 100 new-born babies by Sharma, Handa and Sidhu[12]. Their studies were undertaken to establish the normal levels of free erythrocyte protoporphyrin and erythrocyte coproporphyrin, and to detect at the earliest any error in porphyrin metabolism. The mean levels of free erythrocyte protoporphyrin and coproporphyrin in umbilical cord blood were found to be 74.56 ug/100 ml R.B.C. and 0.34 ug/100 ml R.B.C. with a standard deviation of $\pm$ 28.06 and $\pm$ 0.93 respectively. Relationship between free erythrocyte coproporphyrin and their correlation with period of gestation, birth weight and sex of the new-born were found to be statistically significant.

B. Normal Urinary Porphyrin in Punjabis (Indians)

Urinary porphyrins were determined in 100 normal Punjabis by Raj Kumar and Handa[13,14]. Results are tabulated in table 1.

Urinary uroporphyrins ranged from 0-22.71 ug/24 hours, the mean being 3.75 ug/24 hours with S.D. $\pm$ 3.95. The difference of mean levels of uroporphyrin excretion was statistically significant between the age group 0-14 and 15-40. The difference of mean between the age group 0-14 and 41-70 and also between the age group 0-14 and 15-40 was statistically insignificant. The mean excretion of uroporphyrin in children was found to be lower than in adults. Urinary uroporphyrin excretion per twentyfour hours was more in females than in males. The difference in means in both sexes was statistically insignificant.

The mean uroporphyrin excretion in ug/24 hours was 1.16 in the weight group 41-60 pounds and increased gradually upto 5.09 ug/24 hours in the weight group 121-140 pounds and then decreased in the weight group 141-160 pounds. The mean value in the weight group 101-120 pounds was also less in the weight group 81-100.

No statistically significant difference was observed between the vegetarian and non-vegetarian although the uroporphyrin excretion was higher in non-vegetarian than in vegetarian.

Our values are in contrast with those of El-Mofty et al[15] who observed that excretion of uroporphyrin in 24 hours urine is more in males than in females. The significance of this observation is not clear to us.

Urinary coproporphyrin levels ranged between 12.93-117.85 ug/ 24 hours, the mean being 41.79 ug/24 hours with S.D. $\pm$ 23.26. Effect of age, sex, body weight and diet was studied on urinary coproporphyrin excretion. The relationship of age, sex, body weight and diet to excretion of urinary coproporphyrin is discussed. The difference of mean levels of coproporphyrin excretion was statistically significant

between the age group 0-14 and 15-40. But the difference of mean between the age group 0-14 and 41-70 was statistically insignificant. Mean values of age group 15-40 was also found to be statistically insignificant as compared to the age group 41-70. The correlation between the age and coproporphyrin excretion was statistically insignificant.

The coproporphyrin excretion is more in males than in females. The difference between both sexes is statistically significant.

Urinary coproporphyrin levels showed a positive correlation to body weight, 24 hours coproporphyrin excretion was 26.06 ug in the weight group 21-40 pounds and it increased gradually upto 50.40 ug in weight group of 101-120 pounds. The levels decreased to 49.30 and 39.03 ug/24 hours in the weight groups of 121-140 and 141-160 pounds, respectively. These results were statistically insignificant.

No statistically significant difference was observed between vegetarian and non-vegetarian group. Diet had no effect.

Results of urinary coproporphyrin excretion observed being more in males than in females, are in agreement with those of Zieve et al.[16] Schwartz[17] and El Mofty et al.,[15] who have reported higher values in males than in females.

Our observations of urinary coproporphyrin excretion being proportional to body weight are in agreement with the results reported by Strait[18] et al., and Hsia and Page.[19]

C. Normal Faecal Porphyrin in Punjabis (Indians)

Normal faecal porphyrins were determined by Dass, Handa and Sidhu[20,21]. The results are tabulated in table 1. The amount of faecal coproporphyrin excretion in 100 normal subjects ranged from 0.29 to 3.52 ug/G dry weight of faeces. The value of faecal coproporphyrin in males varied from 0.29 to 3.07 ug/G dry weight with mean of 1.38. Values in females varied from 0.41 to 3.52 ug/G dry weight with a mean value of 1.35 ug/G dry weight. The mean value in male was higher but statistically insignificant. Higher, but statistically insignificant values, were seen in non-vegetarian as compared to vegetarian diet group. In non-vegetarian values ranged from 0.41 to 3.52 ug/G, the mean being 1.44 ug/G dry weight. In vegetarian the values ranged from 0.29 to 2.93 ug/G dry weight, the mean being 1.29. The mean faecal coproporphyrin excretion for the age group 0-14,15-40 and 41-60 varied from 1.01, 1.42 and 1.66 ug/G dry weight respectively.

The value of faecal coproporphyrin increased with age but results were statistically insignificant. The mean value of faecal coproporphyrin excretion gradually increased with increase in body weight from 0.77 ug to 1.96 ug/G dry weight in the weight group 1-10 kg to the weight group above 70 kg. Results were statistically signifiicant.

Faecal protoporphyrin ranged from 1.05 to 14.91 ug/G dry weight and the mean value was 4.77 ug/G dry weight. The amount of faecal protoporphyrin in fifty males varied from 1.15 to 14.91 and in fifty females it varied from 1.05 to 13.68, their mean values being 4.88 and 4.07 respectively. Thus the value was more in males than in females; results were statistically insignificant.

Non-vegetarian had higher faecal protoporphyrin values than vegetarian. Difference of mean was statistically significant. Values in non-vegetarian group varied from 1.05 to 14.91 ug/G dry weight with mean value of 5.64. In vegetarian the values ranged from

1.15 to 7.47 ug/G dry weight with mean value of 3.31. Normal faecal protoporphyrin excretion increased with age. The correlation was positive and statistically significant. The mean faecal protoporphyrin levels in age group 0-14, 15-40 and 41-60 were 2.58, 4.30 and 6.55, respectively. The correlation between the body weight and protoporphyrin excretion was positive and was found to be statistically significant. The mean value in weight group 21-30 was the lowest being 2.21 ug/G dry weight. It was highest in the weight group above 70 kg. being 7.16 ug/G dry weight.

Porphyrin in Disease

Porphyrin levels are abnormally increased in porphyria, a metabolic disorder. Porphyrias can be truly labelled as a Royal malady because medical historians have detected the presence of porphyrin in the Royal members of the Houses of Stuart, Hanover and Prussia. The illness and fits of insanity of King George III, have been traced to porphyria.

Porphyrins can be classified according to Magnus[22] as follows :

TABLE 2
Classification of Porphyrias

A.	Erythropoietic
	Congenital erythropoietic (Gunther's Disease)
	Erythropoietic protoporphyria (or Erythrohepatic protoporphyria)
	Erythropoietic coproporphyria
B.	Hepatic
	Porphyria cutanea tarda (Symptomatic porphyria)
	Acquired
	Familial (controversial)
	Variegate porphyria (South African genetic porphyria, Mixed porphyria)
	Acute intermittent porphyria (Swedish genetic porphyria)
	Hereditary coproporphyria
C.	Classification uncertain
	Hepato—erythrocytic porphyria

Handa[23] reported three cases of Gunther's disease in one family. We subsequently observed two more cases of Gunther's disease and two cases of P.C.T. in Punjabis (Indians). We were unable to classify two cases of porphyria which perhaps belonged to hepatoerythrocytic porphyria. First case of porphyria variegata in an Indian was reported by Handa, Kamlesh Kumar and Raj Kumar[24].

I had the proud privilege of working in the Department of Photobiology with Prof I.A. Magnus and undertook a joint study of estimation of faecal "X" porphyrins with G.H. Elder and Maureen Doyle[25] at the Institute of Dermatology, London, on various porphyrias in White subjects suffering from A.I.P. (five patients), VP (thirteen patients), P.C.T.s (twenty-seven patients) and fifty- two non-porphyric photosensitive control group with miscellaneous dermatoses. The values of faecal "X" porphyrins were significantly higher in cases of porphyrias than in normal subjects, the highest having been found in variegate porphyria (42-910 ug/G dry weight and P.C.T. 0-35 ug/G dry weight).

The research work on porphyrin was extended and this investigative tool was applied to various national and social problems like family planning and leprosy.

Porphyrin study in women on oral contraceptive

Porphyrin estimation in women on oral contraceptive was undertaken and the porphyrogenic effect of oral contraceptive was evaluated. Arora, Handa and Sidhu[26] observed porphyrin levels in forty healthy females by spectrophotometric study. Of these, thirty women who were on oral contraceptives formed the study group, whereas ten women who were on conventional methods of contraception formed

The broad diagnostic features of various types of porphyrias are given below in table 3.

TABLE 3

Diagnostic features of various types of porphyrins

Name	Incidence	Diagnosis
1. Congenital porphyria (Gunther's Disease)	Very rare	All porphyrins (mostly Isomer I) greatly increased in urine, stool and red cells. Red cells show stable fluorescence.
2. Erythropoietic protoporphyria (EPP)	Common dominant transmission	Protoporphyrin increased in red cells, and often in faeces. Urinary porphyrins normal. Red cells show transient fluorescence.
3. Porphyria cutanea tarda (PCT) also known as Hepatic cutaneous porphyria, or Symptomatic porphyria	Common depending on social conditions. Occurs in any race. Rarely familial	Urinary uroporphyrin fraction raised during active phase. Faecal porphyrins may be increased in remission.
4. Variegate porphyria (VP) also known as Mixed porphyria, or South African genetic porphyria	Very common in White Afrikaanders, less common in other races, Familial dominant transmission	Faecal porphyrins increased especially proto and X-porphyrin. Urinary PBC increased during acute attacks.
5. Acute intermittent porphyria (AIP)	Relatively common. Dominant transmission	Urinary PBC greatly increase during and usually between attacks

the control group. It was observed that 66 per cent among the women is the study group, had raised coproporphyrin levels and 16.6 per cent had raised protoporphyrin levels in blood. The levels had no relation to duration for which oral contraceptives were taken. Mean of porphyrin levels in the two groups did not differ significantly as revealed by statistical analysis.

Porphyrin study in leprosy

Porphyrins were studied in various types of leprosy by Pavithran, Handa and Sidhu[27]. An attempt was made to use porphyrin estimation as a sensitive index of hepatic damage due to dapsone (Work under print in International Journal of Leprosy). Quantitative estimations of porphyrins in blood, urine and faeces of thirty patiens with leprosy on treatment with dapsone were done. The results were compared with those of ten untreated cases of leprosy. A comparison was also made with porphyrin levels of normal persons to detect any effect of leprosy on porphyrin levels.

The use of dapsone had no adverse effect on porphyrin metabolism; because none of the cases of leprosy, under study, developed statistically significant raised porphyrin levels of blood, urine and stools. In other words, dose and duration of treatment with dapsone had no porphyrinogenic effect. Raised erythrocytic protoporphyrin may be attributed to the haemolytic effect of the drug.

Although erythrocyte coproporphyrin levels were significantly higher in leprosy patients than in controls and urinary uroporphyrin levels significantly lower, most values fell within the normal range. These differences did not appear to have any clinical significance and the cause remains unknown.

Porphyrin study in lead workers

Occupational hazards of lead workers are well known. This attracted our attention and we made use of this valuable tool of porphyrin estimation for the detection of lead toxicity.

The study was conducted by Mehta, Handa and Sidhu[28] (results unpublished). We undertook to estimate the quantity of porphyrin in blood of thirty lead workers who have been exposed to lead for a period ranging from five years to thirty-two years and compare their erythrocyte porphyrins with that of ten normal persons. These persons, working as monocasters, compositors in printing presses, plumbers and battery repair workers, formed the study group.

This study showed that there was a statistically significant rise of blood protoporphyrins in persons handling lead. However, the correlations with age, diet, body weight, period of exposure to lead and levels of haemoglobin were found to be statistically insignificant. It was observed that there was statistically significant rise in urinary coproporphyrin in the lead workers. There was no statistically significant change in urinary uroporphyrin. However, the correlation with age, diet, body weight, period of exposure to lead, levels of haemoglobin were found to be statistically insignificant.

The range, mean and S.D. for faecal protoporphyrin in study group were 0 to 12.470, 5.512 and 3.184 respectively. The corresponding values for the control group were 2.758 to 9.902, 5.336 and 2.264. By "t" test, the difference was not statistically significant ($t = 0.260$). Faecal coproporphyrins and protopophyrins were not altered to statistically significant level in lead workers. Correlation with age, diet, body weight, period of exposure to lead, levels of haemoglobin were found to be also statistically insignificant.

The tulip fields remind me that the colour of porphyrin is as fascinating as the dazzling colour of these tulips. It beckons you all to join me in the pursuit of our knowledge of porphyrin in Health and Disease.

Finally, I would like to thank you all for this honour bestowed upon me by giving me the opportunity to deliver Dr. Ambady Memorial Oration. I am really beholden to you all.

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