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PORPHYRIAS

SEPARATION AND IDENTIFICATION BY HIGH-VOLTAGE ELECTROPHORESIS

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Porphyrias can be conveniently defined as errors in prophyrin biosynthesis and metabolism, either hereditary or non-hereditary, and often latent.

Porphyrinuria, on the other hand, is the increased excretion of porphyrins in the

Classification of Porphyrias

I. Erythropoietic Porphyrias

1. Hereditary Uroporphyria (M.Guenther)
2. Hereditary Coproporphyria
3. Hereditary Protoporphyria

II. Hepatic Porphyrias

a) congenital forms

1. Acute intermittend Porphyria (Pyrroloporphyria)
   a) manifest form
   b) latent form
2. Porphyria variegata (protocoproporphyria)
   (hereditary south african porphyria)
   a) cutaneous form (without psychic and neurologic manifest.)
   b) abdominal form (mostly psychic alterated)
   c) various combinations
   d) latent form
3. Porphyria cutanea tarda hereditaria
4. Hereditary coproporphyrinuria
5. Purpura porphyrica

b) Acuired forms of porphyrias

Cutaneous hepatic porphyria after intoxication with hexachlorbenzene

c) secondary (symptomatic) porphyrias

1. In Liver disease: cirrhosis, after hepatitis, in lead intoxication, intoxication with sulfonamides, barbiturates, griseofulvin, sedormid and other allylsubstituted pharmakons.
2. In hematologic diseases: iron deficiency, infects, B_6-deficiency, sideroachrestic anemias

Fig. 1. Classification of disturbances in porphyrin metabolism.

urine with or without a disturbance in porphyrin metabolism. Porphyrinuria is therefore a symptom, not a disease—a fact often ignored.

With regard to the clinical aspects, the most important symptoms are related to the nervous system and the skin, the former only occurring in the hepatic porphyrias, the latter both in erythropoietic and in some forms of hepatic porphyrias, as in *variegatta* porphyria and in hereditary and acquired porphyria *cutanea tarda*. The most important skin symptom is photosensitivity resulting in typical light dermatoses on exposed skin surfaces.

The now commonly accepted classification of the various forms of porphyria is demonstrated in Fig. 1.

If any form of porphyria is suspected, one has to prove that an increased excretion of porphyrins in urine or feces or an increased level of porphyrins in erythrocytes actually exists. To demonstrate an increased excretion of urinary porphyrins,

Separation and quantitative determination of porphyrins by high voltage electrophoresis

1. Urine collection for 24 h over ca. 5 g sodiumbicarbonate and in the dark.
2. 100 ml urine are adjusted to pH 7–8 and shaken with 2 g talc (2 times)
3. Talcum layer is washed with 1% sodium acetate
4. Talcum adsorbate is eluted with acetone-HCl (1 n) 9:1
5. Eluates are vacuum dried
6. Dried residue resolve in barbital-EDTA buffer
7. Aliquot of porphyrin concentrate (equiv. to 10 ml urine) is applied to paper- or cellulose acetate strip quantitatively.
8. Electrophoretic separation, pH 8.6 (barbital-EDTA buffer), 600 V/cm, 30–45 min (paper); 20–30 min. (cellulose acetate)
9. Localisation of fluorescent bands (365 nm)
10. Elution of individual porphyrin fractions with 5 n HCl
11. Measure against blank in soret maximum (400–410 nm), 380 and 430 nm
12. Calculation:

a) corrected optical density: $2 \cdot E_{max} - (E_{430} + E_{380}) = d$

for uroporphyrins: $= \frac{10^6 \cdot d \cdot 1.184}{1.000 \cdot 5000 \cdot 20} = d \cdot 5.85$

for coproporphyrin: $= \frac{10^6 \cdot d \cdot 1.040}{1.035 \cdot 5000 \cdot 20} = d \cdot 4.80$

for protoporphyrin: $= \frac{10^6 \cdot d \cdot 1.526}{1.668 \cdot 4890 \cdot 20} = d \cdot 9.38$

5 carboxyl porphyrin: $d \cdot 5.2$
6 carboxyl porphyrin: $d \cdot 5.4$
7 carboxyl porphyrin: $d \cdot 5.6$

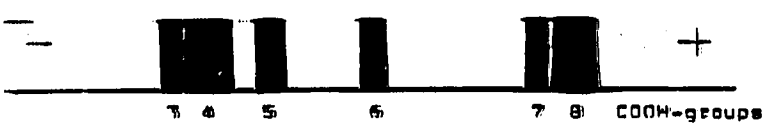

Fig. 2. Procedure for porphyrin analysis.

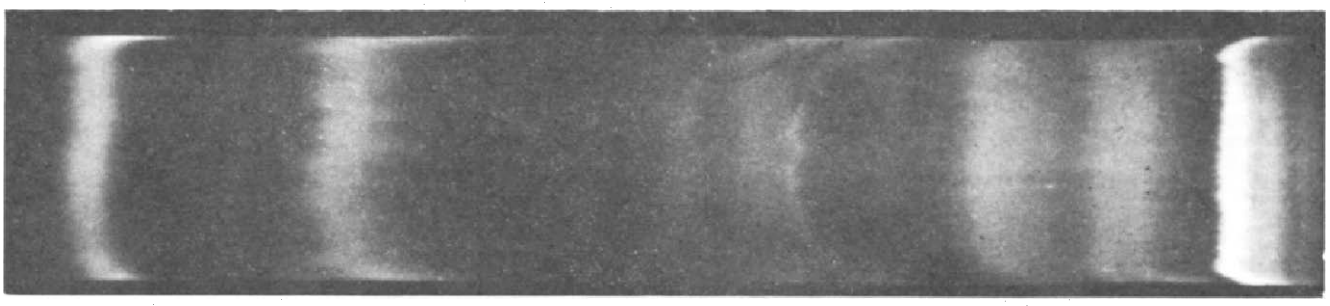

Fig. 3. Separation of porphyrins by high-voltage electrophoresis. (EDTA-barbital buffer pH 8.6; 50–100 V/cm, 10–30 min.)

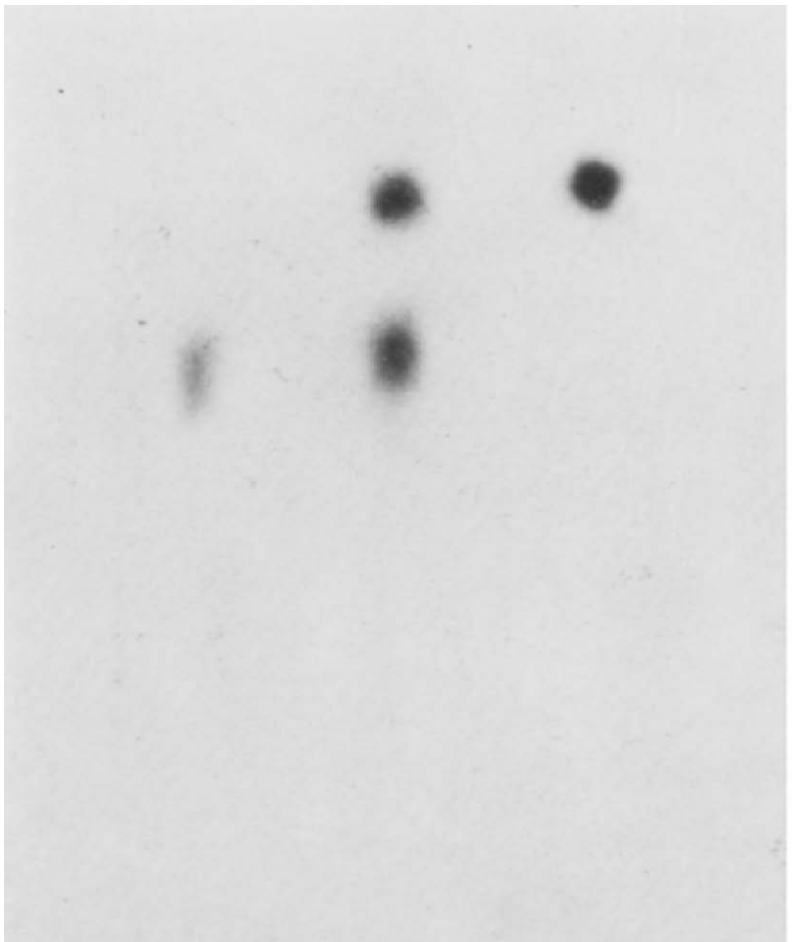

Fig. 4. TLC-separation of coproporphyrin isomers I and III.

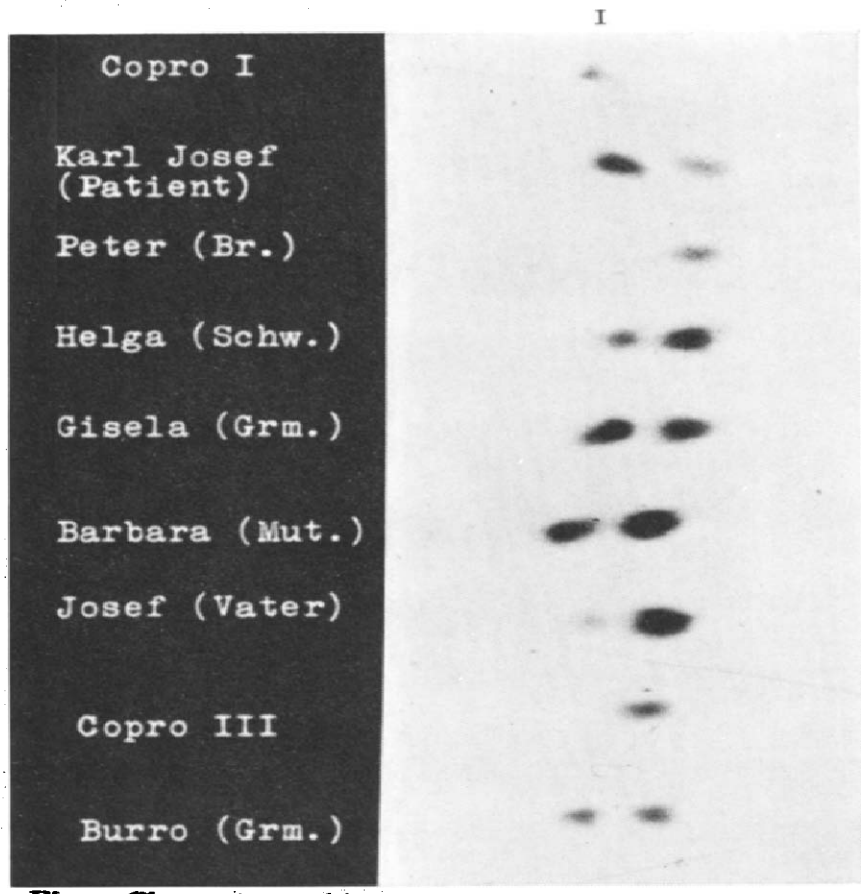

Fig. 5. Chromatographic separation of coproporphyrin isomers in members of a family with congenital uroporphyria (Guenthers disease).

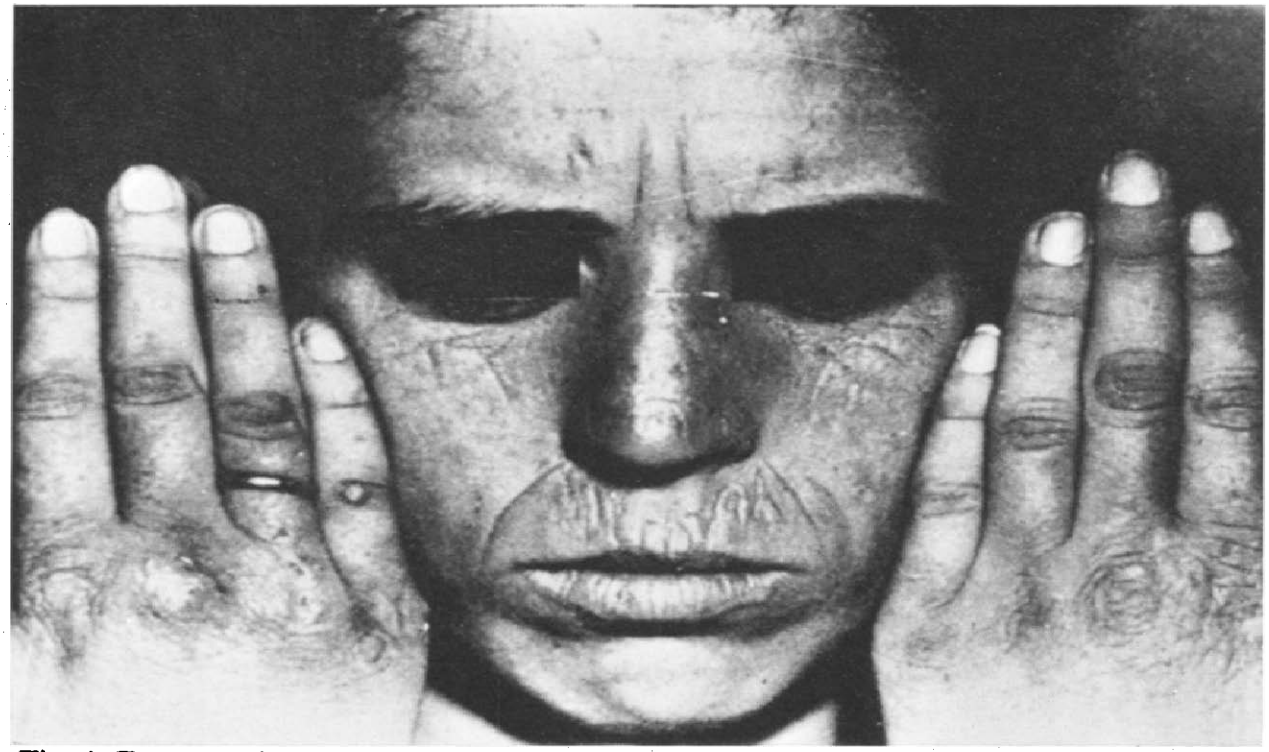

Fig. 6. Protoporphyria.

a very simple extraction procedure can be performed, which serves at the same time as a quantitative analysis if that seems to be necessary.

High-voltage electrophoresis of these extracted porphyrins results in a definite separation into 5–6 bands representing porphyrins with 3 to 8 carboxylic groups.

Normally no protoporphyrin is excreted because it is not sufficiently water-soluble, carrying only two carboxyl groups.

Similar separations are possible with porphyrins extracted from erythrocytes, except that in this case protoporphyrin forms a non-migrating band at the starting point, whereas coproporphyrins and uroporphyrins move to their respective positions. Best results are obtained on Sepraphore cellulose acetate strips, since none of the trailing that is frequently seen on paper occurs, particularly if the porphyrin concentration is very high.

A separation of the individual isomeric types by HVE is impossible, as all isomeric forms of a single porphyrin carry the same net charge. For isomeric separations the electrophoretically separated porphyrin fraction has to be eluted and reseparated under proper conditions by thin-layer chromatography as proposed by Doss and several authors[1,2].

High-voltage electrophoresis of extracted urinary porphyrins and those found in erythrocytes or other biological material not only reveals a picture of the absolute increase of these substances, but also permits a relatively positive identification of the underlying porphyria.

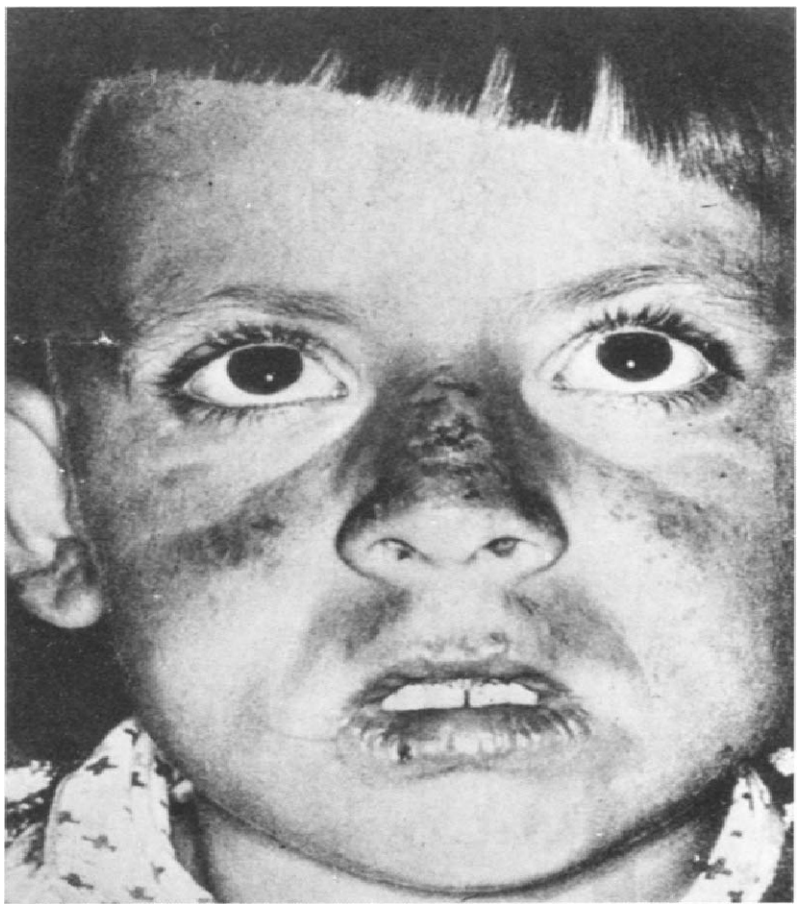

Fig. 7. Protoporphyria.

In the case of erythropoietic porphyrias, only in hereditary uroporphyria (Guenther's disease) can one find significant amounts of porphyrins in the urine besides those immense amounts in the erythrocytes. All porphyrin fractions are present in almost equal concentrations. Separation of isomeric forms reveals a predominance of the isomeric type I, normally present only in traces. This aberration is the real cause of the hereditary defect, an almost absolute deficiency of uroporphyrin isomerase. A few pictures may illustrate the clinical symptoms of this severe but rare disease. The separation of isomers revealed a predominance of the non-physiological type I in various members of the family under investigation.

In the other two forms of erythropoietic porphyrias of dominant inheritance no elevation of urinary porphyrins can be observed, whereas the porphyrin content of erythrocytes is largely increased.

In protoporphyria, first described by KOSENOW AND TREIBS[3] a high amount of protoporphyrin in the erythrocytes (up to several thousand μg) is characteristic for this disease. Similar protoporphyrin concentrations are found in severe lead poisoning, but in this case a large amount of coproporphyrins is excreted in the urine as well as an immense increase of *d*-aminolaevulinic acid. In protoporphyria, however, the urinary porphyrins are quite normal. The same is the case in the third

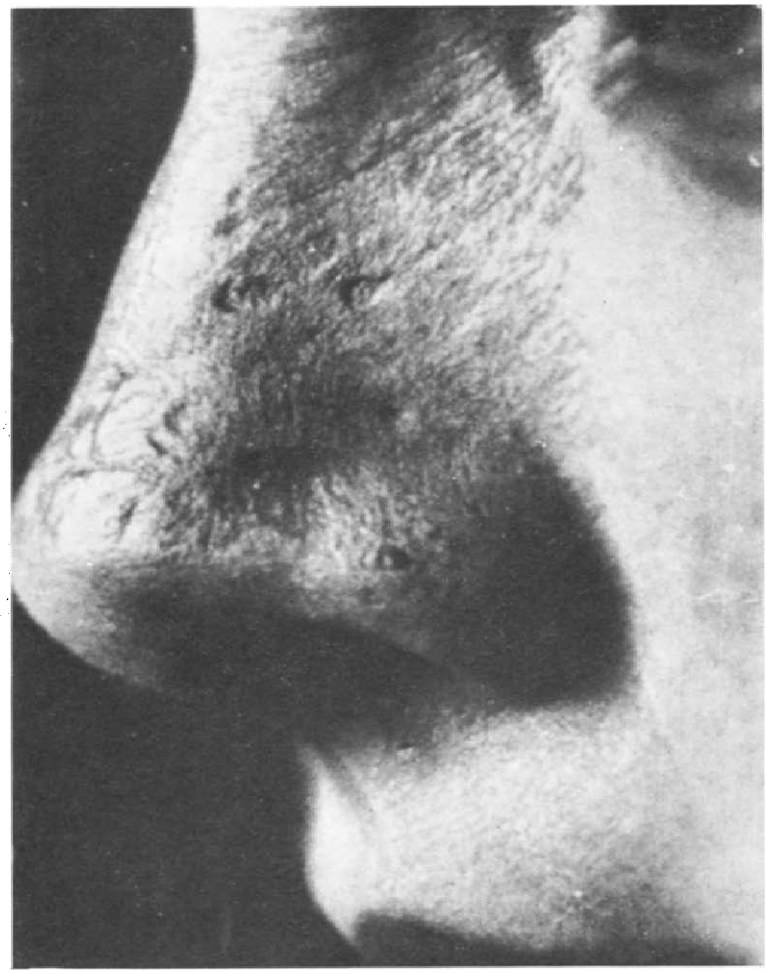

Fig. 8. Coproporphyric light dermatosis.

Fig. 9. Porphyria *cutanea tarda*: separation of urinary porphyrins by high-voltage electrophoresis.

form of an erythropoietic porphyria, coproporphyria[4] (discovered by us a few years ago). It represents the mildest form of porphyric disease caused by moderate to high increase of erythrocyte coproporphyrin of the normal isomeric type III. In this porphyria too, urinary porphyrins are normal in contrast to the relatively rare form of hereditary coproporphyrinuria, where urinary coproporphyrin is very much elevated but erythrocyte porphyrins are within the normal limits.

Most porphyrias are of hepatic origin and of these the so-called porphyria *cutanea tarda* is the most common form. Two different forms exist, the hereditary form and the acquired one. Both have in common the predominance of skin lesions. In the hereditary form, elevated amounts of porphobilinogen and *d*-aminolaevulinic acid as well as acute attacks are frequent. In the non-hereditary form, mostly found in hepatic diseases as for instance in cirrhosis, after severe hepatitis, and in alcoholism, the porphyrin precursors are normal and there are never acute porphyric attacks.

Electrophoresis reveals an excessive predominance of prophyrins with seven carboxylic groups while the uroporphyrin fraction is only moderately elevated. This augmentation of 7-COOH porphyrins is characteristic for this form of a hepatic porphyria and is in strong contrast to the other severe form of hepatic porphyria, the acute intermittent porphyria, in which the uroporphyrins dominate and the porphobilinogen content is excessively elevated.

This can only be a very short summary of porphyric diseases and their clinical and biochemical symptoms. Therefore, it is beyond the scope of this paper to go into further details on porphyrias. I intend only to suggest some experimental possibilities for the revelation and relatively easy identification of some of these metabolic disorders.

REFERENCES

1 M. Doss and W. K. Dormston, *Hoppe Seylers Z. Physiol. Chem.*, 352 (1971) 725.
2 M. Doss and W. Meinhof, *Deut. Med. Wochenschr.*, 96 (1971) 1006.
3 W. Kosenow and A. Treibs, *Z. Kinderheilk.*, 73 (1953) 82.
4 L. Heilmeyer and R. Clotten, in Charles C. Thomas (Editor), *Disturbances in heme synthesis*, Springfield, Ill., 1966.