Increased Fecal Porphyrins in Acute Intermittent Porphyria, *Enrico Rossi* (Biochemistry Section, PathCentre, Queen Elizabeth II Medical Centre, Nedlands, Western Australia 6009, Australia; fax 61 8 9346 3882, e-mail ric.rossi@health.wa.gov.au)

Definitive diagnosis of porphyrias may require determination of total porphyrin in urine, feces, and blood *(1)* and fractionation of the various urine and fecal porphyrins. Most authorities agree that fecal porphyrin fractionation is required for the differential diagnosis of porphyria cutanea tarda, variegate porphyria (VP), and hereditary coproporphyria (HCP) *(1, 2)*. We investigated three patients who excreted excess porphobilinogen (PBG), and who therefore had one of the acute porphyrias. Additional investigation by HPLC fractionation of urine and fecal porphyrins led to a diagnosis of acute intermittent porphyria (AIP). Although total fecal porphyrin in AIP is usually thought to be within the reference interval or slightly increased and of limited assistance in the diagnosis of this type of acute porphyria *(1–3)*, our patients had markedly increased total fecal porphyrins. Fractionation by HPLC revealed that uroporphyrin was a prominent component in all three cases.

Patient A was a woman, 40 years of age, admitted to the emergency department of a tertiary teaching hospital for investigation of intractable acute abdominal pain. Patient B was a woman, 36 years of age, who was initially under the care of an orthopedic surgeon for severe back pain and was referred to a consultant physician for investigation of multiple symptoms, including urinary retention, dysuria, back pain, and depression. Patient C was a woman, 30 years of age, previously diagnosed with AIP at the age of 25 years who consulted a clinician with severe abdominal pain. She had a family history of AIP, and we had identified 15 cases of symptomatic AIP in three generations of her family previously.

We investigated the metabolism of porphyrin by collecting a random urine sample for quantitative analysis of PBG, total porphyrins, and porphyrin fractionation and afecal sample for total porphyrin and porphyrin fractionation. Urine PBG was quantified by anion-exchange chromatography *(4)*, and total urine porphyrin was quantified by fluorometric scanning *(5)*. We corrected both urine PBG and porphyrin results for creatinine concentration by expressing the total concentration as excretion per mole of creatinine *(6)*. Total fecal porphyrin was quantified by a spectrophotometric method, which also provides an acid extract suitable for porphyrin fractionation by HPLC *(7)*. Red cell PBG deaminase activity was determined by spectrophotometry with PBG as the substrate *(8)*.

The porphyrins were fractionated by a modified HPLC gradient system *(2)*, using a Waters 600E pump with flow rate of 1.5 mL/min, a WISP 717 autosampler (Waters), and a 150 × 4.6 mm column packed with 5 μm C_{18} ODS silica (Ultrasphere 235330; Beckman). The gradient started at 100% solvent A (100 mL/L acetonitrile–900 mL/L 1 mol/L ammonium acetate, pH 5.16) and changed through the following step gradient: 50% A, 50% B (100 mL/L acetonitrile–900 mL/L methanol) by 10 min; 20% A, 80% B by 23 min; and 100% B by 24 min. The 100% solvent B step was maintained until the 35-min time point, and then the gradient returned to 100% A by 37 min and was maintained until the 46-min time point to allow for reequilibration. We used fluorescence (not ultraviolet) detection (RF 535; Shimadzu). The excitation wavelength was 395 nm to equalize peak area response for coproporphyrin (392 nm) and uroporphyrin (398 nm), and the emission wavelength was 615 nm.

The results of urinary analyses for PBG, total porphyrins, and the quantitative HPLC fractions are shown in Table 1A. The grossly increased urine PBG excretion concurrent with acute symptoms indicates that an acute attack of porphyria was present. The HPLC traces for patients A, B, and C were very similar, with uroporphyrin being 85%, 78%, and 95% of the total porphyrin, respectively (see Fig. 1A).

Table 1. Urine and fecal porphyrin results.

A. Urine porphyrin	Porphobilinogen, mmol/mol creatinine	Total porphyrin, μmol/mol creatinine	Uroporphyrin, %	Heptacarboxylic porphyrin, % of total porphyrin	Coproporphyrin, %
Reference range	<0.8	<35	5–23	0–4	72–94
Patient					
A	39.5	289	85	3	12
B	27.5	352	78	2	20
C	39.0	637	95	1	4
B. Fecal porphyrin		**Total porphyrin, nmol/g dry wt**	**Uroporphyrin, %**	**Coproporphyrin, % of total porphyrin**	**Dicarboxylic porphyrins, %**
Reference range		<200	<2	2–33	60–98
Patient					
A		350	62	29	9
B		720	23	8	69
C		355	74	17	9

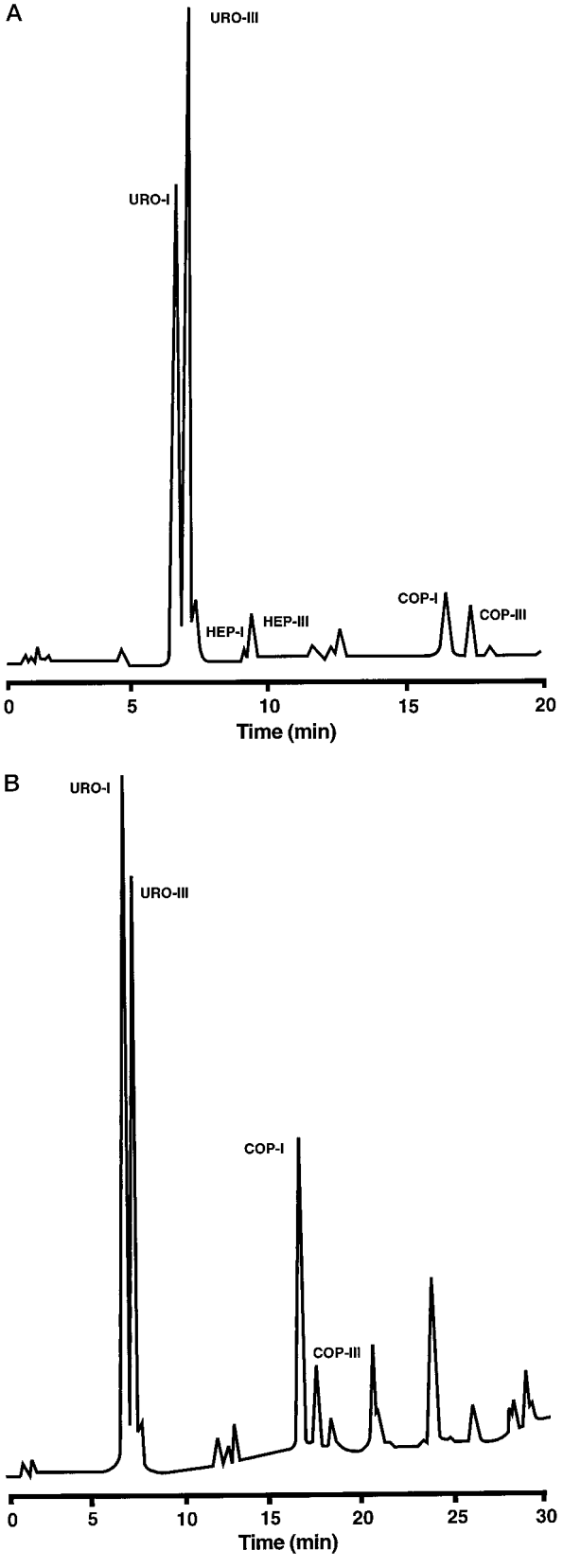

Fig. 1. HPLC of urine (*A*) and fecal (*B*) porphyrins from patient A.

URO-I, uroporphyrin I; *URO-III*, uroporphyrin III; *HEP-I*, heptacarboxylic porphyrin I; *HEP-III*, heptacarboxylic porphyrin III; *COP-I*, coproporphyrin I; *COP-III*, coproporphyrin III.

Results of the fecal analyses total porphyrins, and quantification of the main HPLC fractions are shown in Table 1B. A total fecal porphyrin within the reference range would have been considered by most authorities to be consistent with a diagnosis of AIP *(1–3)*. Fecal porphyrin values were increased (>200 nmol/g dry weight) in all three patients, and fractionation was essential because fecal porphyrin profiles usually provide the differential diagnosis *(1, 2)*. Fecal uroporphyrin was increased in all three patients (Table 1B), accounting for 62% of the total porphyrin in patient A (Fig. 1B), 23% in patient B, and 74% in patient C.

In AIP, measurement of red cell PBG deaminase would be expected to show a 50% reduction in activity *(1, 3)*. A sample of heparin-treated blood was obtained from patients A and C; patient B was not available for follow up. The PBG deaminase activity was 1.64 U/L for patient A and 0.90 U/L for patient C (reference range, 1.20–3.60 U/L), thus confirming AIP in patient C.

Unequivocally increased urine PBG results are diagnostic for one of the types of acute porphyria, usually either AIP, HCP, or VP. An exception to this diagnostic rule is "variant AIP" in malnourished patients receiving anticonvulsant medication *(9)*; however, none of our patients were starved, on diets, or receiving anticonvulsants.

There are difficulties in interpreting PBG deaminase results, because red cell enzyme activity overlaps in healthy individuals and AIP patients, and up to 15% of AIP patients have red cell porphyrin activity within the reference range with decreased activities only in nonerythroid tissue *(3)*. A recent evaluation of the role of this enzyme in the diagnosis of AIP in the US found that 10 of 107 (9%) AIP patients had enzyme activities within the corresponding reference ranges *(10)*. Patient A falls into this category, and the diagnosis of AIP in this patient was based on the fecal porphyrin fractionation results.

The increased urine porphyrin was predominantly uroporphyrin arising from nonenzymatic condensation of micromolar concentrations of PBG to produce a relatively higher concentration of uroporphyrin along with the brown-red pigment porphobilin *(1, 11)*. Correct interpretation of the chromatograms, therefore, requires a prior assay of PBG to correctly identify the origin of the increased uroporphyrin that reflects an acute porphyric attack, which may occur in AIP, HCP, or VP. Although urinary coproporphyrin excretion in HCP or VP usually exceeds that in AIP *(2)*, fractionation of urine is of limited assistance in differentiating these conditions *(1)*.

In the differential diagnosis of porphyria by HPLC profiling, uroporphyrin was described previously as a minor component in feces from patients with porphyrin cutanea tarda in whom urine PBG is not produced *(2)*. In the present three patients, the origin of the fecal uroporphyrin is presumably from nonenzymatic condensation ofPBG. Miyagi et al. *(12)* demonstrated that PBG accumulates in blood during an acute porphyric attack and

reported increased fecal uroporphyrin in two of three patients with AIP and all four patients with VP. Because our three patients were excreting high concentrations of urine PBG at the time of fecal collection, their serum PBG was probably increased. If this is the source of fecal uroporphyrin, it probably would also be seen during acute attacks of the other acute porphyrias, as has been reported for VP *(12)*.

HCP was excluded from the differential diagnosis in all three cases by the low proportion of fecal coproporphyrin III *(2)* and by the proportion of coproporphyrin I exceeding coproporphyrin III for all three cases *(13)*. VP is characterized by a fecal porphyrin trace in which protoporphyrin predominates *(2)* and was excluded because protoporphyrin was <5% of the total porphyrin in all three patients.

We therefore concluded that all three patients have AIP with increased fecal porphyrins and an increased fecal uroporphyrin fraction. The source of the uroporphyrin is probably the nonenzymatic condensation of PBG produced during the acute attack and excreted into the bile. Fecal uroporphyrin in patients excreting excess PBG could, therefore, be a feature of the acute attack in any of the three acute porphyrias.

I thank D. Blake and V. Cronin of the Royal Melbourne Hospital for performing the PBG deaminase determinations.

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Prevention of the Decrease in Sensitivity of an Amperometric Glucose Sensor in Undiluted Human Serum, *Burkhard Linke,[1] Martin Kiwit,[1] Kerstin Thomas,[1] Martin Krahwinkel,[1] and Wolfgang Kerner[2*]* ([1] Department of Internal Medicine, University of Lübeck, D-23538 Lübeck, Germany, and [2] Department of Diabetes and Metabolism, Klinikum Karlsburg, D-17495 Karlsburg, Germany; * address correspondence to this author at: Klinikum Karlsburg, Klinik für Diabetes und Stoffwechselkrankheiten, Greifswalder Strasse 11a, D-17495 Karlsburg, Germany; fax 49-38355-701582, e-mail wkerner@rz.uni-greifswald.de)

The ultimate aim of glucose sensor construction is to provide an accurate means for the measurement of glucose concentrations in vivo as part of a closed-loop insulin delivery system. Various types of sensors (mainly those based on amperometric measurement of hydrogen peroxide generated by enzymatic oxidation of glucose) have been implanted into the subcutaneous tissue of animals *(1, 2)* and human subjects *(3)*. The researchers conducting these experiments routinely found that sensors in subcutaneous tissue had much lower sensitivities to glucose than those in buffer solutions *(3, 4)*.

The causes for this loss of sensitivity in vivo are not known; however, four hypotheses are frequently put forward to explain this phenomenon *(3, 4)*: (*a*) the concentrations of glucose and/or oxygen in the surrounding tissue are lower than expected; (*b*) proteins or other substances are adsorbed onto the outer surface of the sensor, hindering the diffusion of glucose and/or oxygen through the membrane ("membrane fouling"); (*c*) the activity of glucose oxidase is inhibited by an unknown, presumably low-molecular weight substance; and (*d*) detection of hydrogen peroxide by the platinum anode is inhibited ("electrode fouling"). Interestingly, a loss of sensitivity to glucose of similar magnitude is observed in human serum and in serum ultrafiltrate *(3)*. This makes the first hypothesis unlikely, provided that identical mechanisms are responsible for the inactivation of sensors in serum and in subcutaneous tissue. If it were possible to prevent sensor inactivation with a membrane placed between the electrode surface and the enzyme layer, this would provide evidence that the second and third hypotheses are of minor importance and favor, at least indirectly, the importance of the fourth hypothesis. The aim of the present study was to examine the effect of such a membrane on the sensitivity to glucose of a sensor exposed to undiluted human serum for 15 h. The membrane to be tested was from microporous polytetrafluoroethylene (PTFE). This material was chosen because of its known excellent properties in the elimination of interfering electroactive substances *(5)*. The sensor was a commercially available glucose macroelectrode.

Tests were performed with the amperometric flow cell and the multichannel peristaltic pump of the Biostator Glucose-Controlled Insulin Infusion System from Bayer Diagnostic. Each experiment was performed on two different instruments in parallel. The electrode of the flow cell of one instrument (platinum anode, Ag/AgCl cath-