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Plasma Levels of Hemopexin and Albumin in Disorders of Porphyrin Metabolism[1] (38174)

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The biological functions of hemopexin are of increasing interest (1–3). This β-glycoprotein combines with heme in an equimolar ratio and functions in its disposal. Determination of hemopexin levels in patients with hemolytic disease is a simple inexpensive screening procedure for evaluating the severity of hemolysis (4). Hemopexin like albumin binds porphyrins other than heme (5–7) *in vitro* and the possibility of a link between the metabolism of metal-free porphyrins and hemopexin was suggested by the observation that the level of circulating hemopexin is reduced in patients with porphyria cutanea tarda (PCT) (8).

We report the concentrations of plasma hemopexin, albumin and where appropriate, of haptoglobin for patients with 3 aberrations of porphyrin metabolism. Furthermore, we did correlate levels of hemopexin and albumin with those of porphyrins which accumulate in these diseases.

Materials and methods. Several physicians (see Acknowledgment) provided serum or plasma, where possible obtained serially, from 26 patients (33 samples) with acute intermittent porphyria (AIP), 35 patients (103 samples) with erythropoietic protoporphyria (EPP), and 64 patients (67 samples) with PCT. Of the 64 patients with PCT, 14 were patients of Dr. L. C. Harber at New York University; 19 individuals who entered Bellevue Hospital for routine health care served as their controls.

Concentrations of hemopexin, albumin and haptoglobin[3] were measured by radial immunodiffusion (9). Total porphyrin (>90% protoporphyrin) in erythrocytes, plasma (10) and feces (11) was determined in 76 samples from 14 patients with EPP by Dr. M. A. Pathak of Harvard University. Concentrations of uro– and coproporphyrin were assessed in the urine of 20 patients with PCT.

Results are the arithmetic mean ± S.E.M., and the means were compared by the Student's t test. To obtain the value of P, a degree of freedom $N_1 + N_2 - 2$ was used throughout, and P values of <0.05 were considered significant.

Results. Plasma hemopexin values of 26 patients with AIP (under care of Dr. L. Wetterberg (12)) fell within the range observed for normal adults, i.e., 50–100 mg/100 ml (1), with the exception of four whose hemopexin level was reduced to 26, 31, 44, 49 mg/100 ml respectively. The

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[3] Determined in the laboratory of Dr. T. S. Edgington, Scripps Clinic and Research Foundation, La Jolla, California.

mean albumin concentration for all patients is listed in the Table I. It is lower than that of control subjects. Individual patients showed a direct correlation between values for hemopexin and albumin ($r = 0.705$, $P < 0.001$, $n = 33$). However, several severely ill patients had normal hemopexin and albumin levels. The mean haptoglobin concentration for 11 patients (18 samples) was 91.7 ± 20.5 mg/100 ml which is lower than that found for healthy adults (mean 168.8 ± 3.8 mg/100 ml). No porphyrin data were available for these patients.

Hemopexin concentrations ranged from 22 to 49 mg/100 ml in 15 of 35 patients with EPP; the remaining 20 patients had values in the normal range. The mean hemopexin value for the whole group was lower than that of the control population (Table I). Although the albumin values were not always reduced, a direct correlation was found between levels of albumin and hemopexin for 99 samples from the 35 patients ($r = 0.213$, $P < 0.05$). Haptoglobin levels varied from 32 to 288 mg/100 ml (10 patients, 18 samples) with a mean value of 135.2 ± 29.7 mg/100 ml which is somewhat lower than the normal mean 168.8 ± 3.8 mg/100 ml. However, for individual patients, an inverse correlation existed between hemopexin and haptoglobin levels ($r = -0.550$, $P < 0.02$, $n = 18$). Significant correlations were obtained for 14 patients with EPP between the concentrations of hemopexin and the amounts of protoporphyrin in erythrocytes ($r = -0.330$, $P < 0.01$, $n = 71$), stool ($r = -0.569$, $P < 0.001$, $n = 60$), and plasma ($r = 0.370$, $P < 0.05$, $n = 32$). Similarly, an indirect correlation was noted for albumin concentrations and protoporphyrin content of stool ($r = -0.238$, $P < 0.05$, $n = 60$), but neither erythrocytes ($r = 0.155$, $P > 0.1$, $n = 71$) nor plasma ($r = 0.075$, $P > 0.1$, $n = 32$).

TABLE I. Plasma Concentrations of Albumin and Hemopexin in Control and Porphyric Subjects.

	Albumin g/100 ml (±SEM)	Hemopexin mg/100 ml (±SEM)
Control Subjects (30)	4.27 ± 0.14	69.3 ± 2.0
AIP (26)	3.48 ± 0.17[a]	66.5 ± 3.3
EPP (35)	3.77 ± 0.12[b]	57.4 ± 2.8[b]
PCT (64)	3.49 ± 0.085[a]	52.9 ± 1.8[a]

[a] Difference from control population, $P < 0.001$.
[b] Difference from control population, $P < 0.01$.

Twenty-seven of 64 patients with PCT had low values of plasma hemopexin, ranging from 26 to 49 mg/100 ml. Normal hemopexin values were found in the remaining patients as well as in the 19 control subjects of Bellevue Medical Center (73.0 ± 2.5 mg/100 ml). Both mean hemopexin and albumin values for all 64 patients were much lower than those of the control population (Table I). A significant positive correlation existed between values of albumin and hemopexin for individual patients ($r = 0.490$, $P < 0.001$). The mean haptoglobin level for 36 patients (40 samples) was slightly reduced (123.7 ± 11.3 mg/100 ml vs 168.8 ± 3.8 mg/100 ml). For the 20 patients with PCT in whom porphyrins were determined, an inverse correlation was found between the amount of urinary coproporphyrin and the levels of hemopexin ($r = -0.436$, $P < 0.05$, $n = 24$) but not of albumin ($r = 0.108$, $P > 0.05$, $n = 24$). The concentrations of both proteins did not correlate with the urinary uroporphyrin content.

Discussion. Information on aberrations of the enzymatic pathways of porphyrin formation and catabolism is scarce (13), and little is known about porphyrin-binding plasma proteins which may engage in their metabolism (3). In the present study, plasma levels of albumin and hemopexin, the 2 porphyrin binders; and haptoglobin, the hemoglobin binder, were measured in AIP, EPP and PCT. Patients with all three porphyrias had diminished mean albumin and haptoglobin concentrations, whereas those of hemopexin were lowered only in EPP and PCT. Values for hemopexin and haptoglobin did not correlate, and the lowered levels of these 2 proteins are therefore not due to hemolysis. At present, no explanation can be offered for the variable reduction in concentrations of these three

plasma proteins, which are synthesized in the liver (2, 3, 14). But, decreased synthesis rates as well as increased catabolic rates should be considered. In turnover studies with ^{125}I-hemopexin, 5 patients with EPP and one patient with PCT showed normal synthesis rates but increased rates of catabolism (15). The prolonged existence of an abnormally high porphyrin content of the liver in human porphyrias could be responsible for decreased hemopexin and albumin synthesis, and the degree of alteration in liver function, a prominent feature of both EPP and PCT (13), must be taken into account in further investigations.

Correlations were sought between levels of both hemopexin and albumin and porphyrin levels in EPP and PCT. Patients with EPP accumulate abnormally high amounts of protoporphyrins in red cells and feces, and patients with PCT excrete large amounts of uroporphyrin into urine and feces (13). In EPP, values of both proteins correlated inversely with protoporphyrin content in feces. In PCT, hemopexin levels correlated inversely with urinary amounts of coproporphyrin; but this porphyrin is not pathognomonic. Hemopexin and albumin may be instrumental in the disposal of certain non-metal containing porphyrins in a manner analogous to that of heme (1–3). However, a positive (direct) correlation exists between hemopexin, but not albumin levels, and protoporphyrin and coproporphyrin content of erythrocytes and liver in mice made porphyric by ingesting griseofulvin for a few weeks. Besides developing hyperhemopexinemia, they invariably grew hepatomas.[4] Measurement of hemopexin and albumin concentrations and studies of their turnover in patients with porphyrias may increase our understanding of the role porphyrin-binding proteins have in porphyrin metabolism.

Summary. We report on plasma levels of the porphyrin-binding proteins, hemopexin and albumin for 125 patients with three porphyric diseases. Hemopexin concentrations fell within the low normal range in patients with AIP. They were diminished in approximately half of the patients with PCT and EPP. In some of these patients, haptoglobin levels were also measured and found to be lowered, although not concomitantly with those of hemopexin. Albumin concentrations were decreased in many patients with either of the three porphyrias, but the extent of diminution was not invariably associated with a decrease in plasma hemopexin. A highly significant inverse correlation existed between levels of hemopexin and fecal protoporphyrin content in patients with EPP. These findings suggest an interrelationship between the metabolism of hemopexin and albumin and nonmetal containing porphyrins.

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[4] As studied by Dr. D. J. Cripps, Division of Dermatology, University of Wisconsin Medical Center, Madison, Wisconsin.

1. Muller-Eberhard, U., New Engl. J. Med. 283, 1090 (1970).

2. Muller-Eberhard, U., Liem, H. H., *in* "Plasma Proteins" (A. C. Allison, ed.), Plenum Press, London (1974).

3. Muller-Eberhard, U., and Morgan, W. T., Ann. N. Y. Acad. Sci. (1974).

4. Eyster, M. E., Edgington, T. S., Liem, H. H., and Muller-Eberhard, U., J. Lab. Clin. Med. 80, 112 (1972).

5. Koskelo, P., Toivonen, I., and Rintola, P., Clin. Chim. Acta 29, 559 (1970).

6. Morgan, W. T., and Muller-Eberhard, U., J. Biol. Chem. 247, 7181 (1972).

7. Seery, V. L., and Muller-Eberhard, U., J. Biol. Chem. 248, 3796 (1973).

8. Meiers, H. G., and Ippen, H., Klin. Wschr. 46, 560 (1968).

9. Mancini, G., Carbonara, A. O., and Heremans, J. F., Immunochemistry 2, 235 (1965).

10. Schwartz, S., Berg, M. H., Bossenmaier, I., and Dinsmore, H., *in* "Methods of Biochemical Analysis" (D. Glick, ed.), Vol. 8, p. 221. Interscience Publishers, Inc., New York (1960).

11. Holti, G., Rimington, C., Tate, B. C., and Thomas, G., Quart, J. Med. 27, 1 (1958).

12. Wetterberg, L., "A Neuropsychiatric and Genetical Investigation of Acute Intermittent

Porhyria" (thesis). Lund, Svenska Bokförlaget/ Norstedts (1967).

13. Marver, H. S., and Schmid, R., *in* "The Metabolic Basis of Inherited Disease" (J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, eds.), Third ed., p. 1087. McGraw-Hill, Inc., New York (1972).

14. Hamashima, Y., Harter, J. G., and Coons, A. H., J. Cell Biol. **20**, 271 (1964).

15. Wochner, R. D., Spilberg, I., Iio, A., Liem, H. H., Muller-Eberhard, U., New Engl. J. Med. **200**, 822 (1974).

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