PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 647–651 (1978)

Human Histidine-Rich Glycoprotein. II. Serum Levels in Adults, Pregnant Women and Neonates (40265)

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Histidine-rich glycoprotein (HRG)[5] is a 3.8S α_2-glycoprotein of human plasma first described in 1972 (1); its function remains unknown. The peptide portion of this glycoprotein, which has a molecular weight of 58,000 and contains two noncovalently linked subunits, has 9% by weight histidine. Investigation of the biological and physicochemical properties of this protein was undertaken after its ability to bind heme and certain nonferrous divalent metal ions was recognized (2). As a first step in establishing the physiological function of HRG and the pathophysiological consequences of altered HRG metabolism, a survey of the serum concentrations of this protein in different age groups in health and disease was undertaken. Because of the ability of HRG to bind heme, the disease states chosen for this initial screening included various hemolytic and porphyric disorders. Of special interest is the steady decline of HRG concentrations in the serum of pregnant women during the last two trimesters of pregnancy.

Materials and methods. Sera of nine newborns, 100 healthy adults, 140 pregnant women, and of 275 individuals with a wide variety of illnesses and 566 cord serum samples were examined. Drs. Ursula Muller-Eberhard and Sallie Hoch, Scripps Clinic and Research Foundation, La Jolla, CA, kindly made available a large number of samples from their serum collections. Many of the sera analyzed were small portions of blood samples drawn for routine laboratory analysis, e.g. Rh factor testing in Rh negative women. Several healthy adults volunteered to provide small serial blood samples. Informed consent forms were obtained in each applicable case.

HRG was purified from human serum by methods modified from those of Heimburger *et al.* (1), and goat immune serum of high titer against HRG was prepared and characterized as described (2). Both antigen and normal human serum gave a single precipitin line of identity when tested against anti-HRG by the Ouchterlony technique (3). Concentrations of purified HRG in standard solutions were determined spectrophotometrically, using an $E^{1\%}_{280}$ of 5.85 (1).

The amount of HRG in serum was measured by radial immunodiffusion (4) using 1% agarose plates with 10 m*M* veronal buffer, pH 8.6, and 3% polyethylene glycol (PEG 6000). Samples (10 μl) were examined in duplicate or triplicate, and standards were applied in duplicate on each plate. Sharply delineated precipitin rings were observed, and the serum values were obtained from the linear calibration curve constructed from standard solutions of purified HRG. Standard curves made in California, Michigan and Finland were in good agreement.

Some loss of antigenicity of HRG to repeated freezing and thawing was noted early in this work. Although this could be obviated by quickly freezing the solutions with liquid nitrogen, we prepared quick-frozen aliquots of HRG which were thawed, used once and then discarded. In serum, HRG seems much more stable to repeated freezing and thawing. Nevertheless, to avoid possible denaturation of HRG in the serum samples, they were subjected to a minimum of freeze-thaw cycles and analyzed when possible within 24 hr after sampling. The standard error of determina-

[5] Abbreviations used: HRG, 3.8S α_2-histidine-rich-glycoprotein; heme, iron-protoporphyrin IX.

0037-9727/78/1584-0647$01.00/0

tions in the concentration range 2.5–30 mg/100 ml was found to be ±8% (n = 25). Statistical calculations were made with an SR-52 calculator and programs from Texas Instruments. Differences between the HRG levels of groups of subjects were tested by Student's t test.

Results. The concentration of HRG in healthy adult serum was determined to be 12.5 ± 3.2 mg/100 ml, mean ± SD, n = 100 (Table I). This is higher than the 9.2 ± 4.5 mg/100 ml reported in the first description of HRG (1). We obtained similar results using Behringwerke HRG, kindly supplied by Dr. G. Schwick, or our own HRG as standards. The same HRG concentration was found in serum, plasma and defibrinated plasma samples of several healthy adults tested.

No differences between the mean HRG levels in healthy adults of different ages, nor between males and females was noted (Table I). The HRG levels in the sera of healthy neonates was determined to be 2.3 ± 1.5 mg/100 ml, n = 9 (Table II), significantly lower than adult levels, $P < 0.005$. The HRG concentrations determined in nearly 600 cord serum samples (Table II, 3.4 ± 1.1 mg/100 ml) showed a strong positive correlation (r = 0.90) with increasing time of gestation but only a weak correlation (r = 0.23) with the weight of the infant at birth. These correlations were determined using a least squares linear regression program. The levels of HRG in nine pairs of nonidentical twins were similar (r = 0.72). A value of 3.45 mg/100 ml for HRG concentration in neonatal serum was previously reported (1).

TABLE I. HRG CONCENTRATIONS IN NORMAL HUMAN SERUM OF ADULTS.

Group[a]	Number of individuals	HRG (mg/100 ml) Mean	Range	SD
Males	55	12.8	7.4–24.8	3.5
Females	45	12.0	6.8–19.2	2.7
Total	100	12.5	6.8–24.8	3.2

[a] The ages of these subjects ranged from 21 to 57 years, with a mean of 35 and a SD of 9.9 years. There was no correlation of HRG with age in this population. Females with hysterectomies, taking oral contraceptives, or with zero to five children, exhibited HRG levels in the normal range.

TABLE II. HRG CONCENTRATIONS IN PAIRED MOTHER AND INFANT SERA AND IN CORD SERUM.

Category	Number of individuals	HRG (mg/100 ml) Mean	Range	SD
Mothers	9	4.2	2.5–5.9	1.0
Infants	9	2.3	1.0–4.4	1.5
Cord serum				
Males	325	3.5	—	1.2
Females	241	3.3	—	1.2
Total	566	3.4	0.8–7.4	1.1
Caesarian section infants	96	3.2	—	1.2
Time of gestation				
≤ 35 weeks	14	1.8	0.8–3.5	0.5
36–38 weeks	116	2.8	1.0–5.9	1.0
39–41 weeks	370	3.6	1.0–7.0	1.2
≥ 42 weeks	60	4.3	2.0–7.4	1.3

TABLE III. HRG CONCENTRATIONS IN WOMEN DURING PREGNANCY, AT TERM, AND POSTPARTUM.

Subject category[a]	Number of individuals	HRG (mg/100 ml) Mean	Range	SD
Therapeutic abortion, 8–12 weeks	21	10.7	8.3–12.5	1.6
One day prepartum	14	5.6	3.0–8.0	1.6
At parturition	93	5.6	1.5–9.3	2.3
1–3 days postpartum	21	6.0	2.3–11.3	2.8
4–15 days postpartum	8	11.8	9.5–12.8	3.3

[a] Each sample set represents a different group of women.

The low HRG concentrations in the mothers of infants shown in Table II led us to examine this phenomenon in more detail. The sera of women at term (Table III) contain significantly lower concentrations of HRG (5.6 ± 2.3 mg/100 ml, $P < 0.005$), than those of healthy adults. The sera of women who received therapeutic abortions before the 12th week of pregnancy contained concentrations of HRG only slightly lower than normal (Table III). The time course of the decrease in HRG levels during pregnancy in five women is shown in Fig. 1. A gradual, almost linear decline commences near the start of the second trimester of pregnancy and progresses to about 50% of the mean adult HRG concentration. The levels of HRG appear to return to normal by 5–15 days postpartum (Table III, Fig. 1).

A survey of the HRG levels in the serum of patients with a wide range of disorders was started to explore the diagnostic usefulness of this protein and to search for clues to its physiological function. The results of this work to date is summarized graphically in Fig. 2. No significant correlations between

the concentrations of HRG and other serum proteins such as haptoglobin, hemopexin, transferrin, albumin and the third component of the complement system (C3) were detected in any of the sera for which datum was available (data not shown). The mean values of HRG levels found in most of these disease categories are not significantly different from that of healthy adults ($P > 0.1$), although the scatter is greater. No change in HRG levels was noted in the serum of patients with hemolytic diseases (Fig. 2) or of several porphyric patients and normal volunteers during the course of intravenous infusions of heme (data not shown).

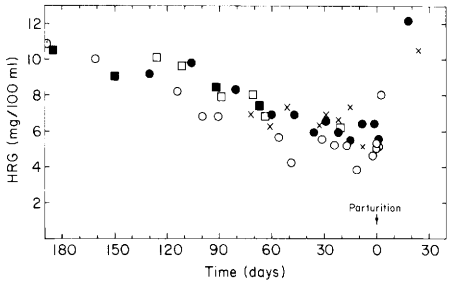

FIG. 1. Concentrations of HRG in serum of pregnant women. The levels of HRG were determined in serum samples of five women at various times during the course of their pregnancies. The actual date of delivery was used to determine the time for subjects (○, ●, ×). The expected delivery date was used for one subject who suffered a miscarriage three weeks before expected parturition (□) and for another subject (■) who had not given birth at this writing. One subject (×) delivered one month before the expected date.

However, certain groups are of interest. First, several patients with a variety of heart ailments, including myocardial infarction, rheumatic heart disease and heart valve surgery, showed elevated HRG concentrations (16.6 ± 6.2 mg/100 ml, $n = 36$, $P < 0.01$). Treating this variety of heart conditions as a group is not intended to imply specific effects but rather to point to an observation which suggests further study. Second, patients with erythropoietic protoporphyria (14.5 ± 5.9 mg/100 ml, $n = 18$) had higher ($P < 0.05$), and those with porphyria cutanea tarda (11.5

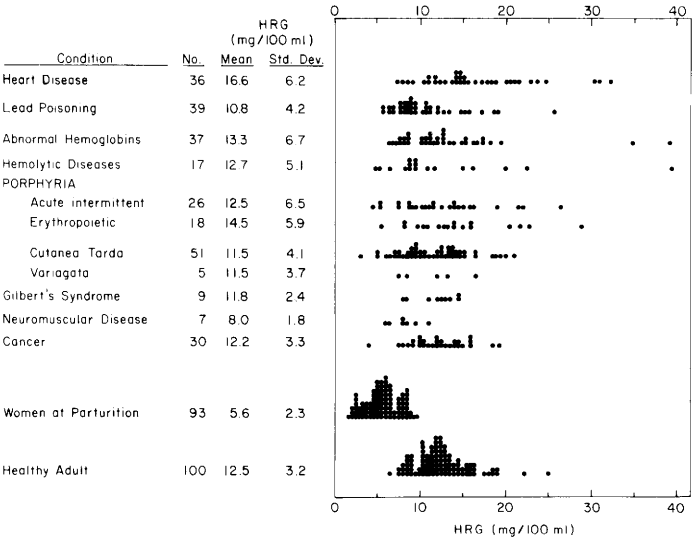

FIG. 2. Concentrations of HRG in serum. The levels of HRG were measured in serum samples of healthy adults, women at parturition and groups of subjects with various classes of illness. The heart disease group included patients with myocardial infarction, rheumatic heart disease, and congestive heart failure patients, as well as patients who had heart valve surgery; the lead poisoning group, patients with both acute and chronic lead exposure; the abnormal hemoglobin group included patients with β-thalassemia, thalassemia trait, and unstable hemoglobins; the hemolytic disease group included patients with autoimmune hemolytic anemia, hemolytic anemia and paroxysmal nocturnal hemoglobinuria; the neuromuscular disease group included patients with spastic paraplegia and myasthenia gravis; the cancer group included patients with Hodgkin's disease, with carcinoma (some with metastases) of the colon, ovary, lung, liver, breast, and with lymphocytic lymphoma, lymphosarcoma and three patients with Waldenström's macroglobulinemia.

± 4.1 mg/100 ml, $n = 51$), lower ($P < 0.05$) levels of HRG than normal. In some of the patients with porphyria, lower values of HRG were noted to correspond to high levels of porphyrins excreted into the urine. Third, the three patients in the cancer group with Waldenström's macroglobulinemia exhibited depressed HRG levels (6.8 ± 2.4 mg/100 ml) but statistical treatment is not possible with this limited number of cases. Other groups of patients which displayed levels of HRG that were statistically different from healthy adult levels were the neuromuscular disease (8.0 ± 1.8 mg/100 ml, $n = 7$, $P < 0.05$) and lead intoxication (10.8 ± 4.2 mg/100 ml, $n = 39$, $P < 0.05$) groups (Fig. 2). Although the mean HRG concentration measured in the early stages of this work in a set of serum samples from female relatives of patients with Duchenne's muscular dystrophy (10.2 ± 2.2 mg/100 ml, range 5.0–14.5 mg/100 ml, $n = 100$) was less than the healthy adult mean ($P < 0.01$), incomplete information on the age and pregnancy status of the subjects was available. Since most of the lead poisoning cases were juveniles, the decreased mean HRG level may represent a normal lower level of HRG in this age group.

Discussion. The biological function of HRG remains unknown, although recent work (2) and the data reported here suggest some lines of investigation that merit attention. The high content of histidine, near 9% by weight of the peptide portion of HRG, may provide a clue to its function. Histidine is well-known to be an axial ligand to heme iron in many heme proteins and to participate in metal ligation in several copper- and zinc-containing enzymes and proteins (5). Human serum HRG has been shown *in vitro* to bind divalent zinc and copper and up to 10 heme molecules with a Kd near 10^{-6} *M* (2). This affinity is too low to make it likely that HRG is essential for heme transport *in vivo*, since human serum albumin (Kd < 10^{-8} *M*, 6) and hemopexin (Kd < 10^{-12} *M*, 7) have greater affinities for heme and occur at significantly higher concentrations in serum. This expectation is supported by the failure of HRG to compete successfully with albumin for heme *in vitro* (2) and by unaltered HRG levels in hemolytic diseases (Fig. 2) and in porphyric patients undergoing heme therapy (data not shown).

The decline of HRG during pregnancy is not unique, as both increased and decreased levels of many serum proteins during pregnancy have been observed (8). However, this decline does indicate that HRG metabolism is altered during pregnancy and may be a clue to its biological function. For example, if HRG functions in the transport of essential metals like zinc, the lower levels may be due to an increased metabolic activity of HRG needed to meet the nutritional demands of the fetus. Changes in plasma zinc content have been observed in pregnancy (9). This possibility is being examined in animal model systems. Of course, other explanations are possible. For example, there may be hormonal effects, although the lack of difference between the HRG levels of males and females, both adult and neonatal (Tables I and II), including some women after hysterectomy and some taking oral contraceptives, and the slow decline in HRG concentrations during pregnancy seems to argue against this idea. Differences from the observed lowering of HRG concentrations during normal gestation may be indicators of some pathological conditions. At present this is being examined in more detail, especially with regard to essential metal metabolism and toxic metal effects since HRG binds divalent copper, zinc, nickel and cadmium (2).

The altered concentrations of HRG in some of the diseases examined (e.g. heart ailments and porphyric diseases) may point to the usefulness of monitoring HRG levels in these patients as an additional means of assessing the severity and course of these diseases. In this regard, it is interesting that altered concentrations of total plasma zinc and zinc bound to lower molecular weight (<80,000 daltons) plasma proteins have been reported to correlate with the severity of several disease states (10–12). In these studies, the zinc–protein complexes were not directly identified, and the possible contribution of zinc–HRG complexes to this finding merits examination. It should be pointed out that HRG is not related to the previously described serum zinc-α_2-glycoprotein (13). The role of HRG and the clinical applications of monitoring HRG levels in disease states suggested by this work are currently being investigated.

Summary. Concentrations of 3.8S histi-

dine-rich glycoprotein were determined in the sera of healthy adults, pregnant women, neonates, and of persons afflicted with a variety of diseases. Quantitative differences were found between the HRG concentration in healthy adult serum (12.5 ± 3.2 mg/100 ml, mean ± SD) and in neonatal (2.3 ± 1.5 mg/100 ml) and cord serum (3.4 ± 1.1 mg/100 ml). In women the HRG concentration declines steadily during the last two trimesters of pregnancy reaching at parturition a value about 50% of that in adult serum (5.6 ± 2.3 mg/100 ml, $P < 0.005$), but returns to normal levels within 5–15 days postpartum. No difference was observed between the HRG levels of males and females in either adults or neonates.

In general, mean HRG levels in the sera of patients with a variety of disease states were near normal but were more widely scattered than in healthy adults. However, the mean HRG concentrations in the sera of patients with a variety of heart ailments (16.6 ± 6.2 mg/100 ml, $P < 0.01$) and erythropoietic protoporphyria (14.5 ± 5.9 mg/100 ml, $P < 0.05$) were elevated compared to healthy adult levels. The levels of HRG in the sera of patients with neuromuscular diseases (8.0 ± 1.8 mg/100 ml), lead poisoning (10.8 ± 4.2 mg/100 ml), and porphyria cutanea tarda (11.5 ± 4.1 mg/100 ml) were lower than normal levels ($P < 0.05$).

The advice of Dr. Ursula Muller-Eberhard and the expert technical assistance of Ms. Kristie Forrest and Mr. Roger P. Sutor is gratefully acknowledged. This work was supported by a grant from the National Institutes of Health (HD-09252). Dr. William T. Morgan is the recipient of a Research Career Development Award (AM-00110).

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Received April 12, 1978. P.S.E.B.M. 1978, Vol. 158.