

Plasma Concentrations of Connective Tissue Compounds: I. The Effect of Uterine Involution (39211)

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Hydroxyproline is one of the amino acids which makes the structure of collagen unique. It is customary to estimate collagen on the basis of the quantitative measurement of its hydroxyproline content. LeRoy *et al.* (1) demonstrated a hydroxyproline-containing protein in human plasma and called it "hypro-protein," suggesting that it may be circulating plasma collagen. Clq, one of the plasma glycoproteins, is the first component complement. It has a unique structure because it contains a collagen-like peptide sequence. The overall composition of Clq resembles that of basement membrane proteins, although Clq does not contain 3-hydroxy but only 4-hydroxy proline (2). Hydroxyproline accounts for 5.42% of the molecular weight of Clq (3). LeRoy therefore subsequently assumed that the protein-hydroxyproline in the plasma (hypro-protein) is due to the circulating Clq (4).

The present study was performed to determine whether there are significant changes of circulating connective tissue components and of Clq in the plasma when extraskelatal connective tissue is increased or during its rapid breakdown.

Material and methods. Pregnant women were prospectively followed during the third trimester of pregnancy (a period of increased extraskelatal connective tissue), during the fourth postpartum day (a period of rapid extraskelatal connective tissue hydrolysis), and six weeks postpartum (normal baseline period). The patients were nine healthy young women, the age range 16 to 23, seven were black and two white. All had a normal antepartum course and were seen in the Prenatal Clinic on regular intervals. All pregnancies resulted in a normal healthy infant, except in one case of an intrauterine fetal death at term. This death was due to

cord strangulation resulting in anoxia of the infant. All patients had a normal postpartum course and were discharged from the clinic following their 6-week check up. EDTA plasma was collected after an overnight fast. Plasma collagen was determined by the method of Bergman and Loxley (5), Clq according to Mancini *et al.* (6), by immunodiffusion.¹ The collagen-hydroxyproline was estimated by subtracting from the total protein-hydroxyproline, the hydroxyproline due to Clq, assuming 5.42% of immunoreactive Clq is hydroxyproline (3). Plasma-free proline was measured by the method of Troll and Lindsley (7), and glycosaminoglycuronans (GAG) were precipitated in 70% ethanol from trichloroacetic acid supernatants of pronase digested 2-ml aliquots of plasma and were measured by methods previously reported (8).

Results. Table I describes the respective values for the third trimester, fourth day postpartum, and 6 weeks postpartum. Plasma Clq remained unchanged at all three periods. There were no differences between the plasma protein-hydroxyproline concentrations obtained during the third trimester of pregnancy compared with those obtained 6 weeks postpartum. During the third trimester and 6 weeks postpartum, essentially all the plasma protein-hydroxyproline was accounted for by Clq. However, there was a striking increase of plasma protein-hydroxyproline (probably collagen) concentrations during the fourth postpartum day without changes of corresponding Clq at the same time.

Plasma-free proline concentrations were also similar during the third trimester and 6

¹ The anti serum was obtained from Behring Pharmaceuticals.

TABLE I. THE EFFECT OF CHANGES OF EXTRASKELETAL CONNECTIVE TISSUE IN PLASMA (MEAN \pm SE).

	Pregnancy		Postpartum	
	3rd trimester		4th day	6 weeks
Protein HO-proline (mg/dl)	1.05 \pm 0.11		2.06 \pm 0.14	1.23 \pm 0.69
		$P < 0.001$		$P < 0.001$
Clq HO-proline (mg/dl)	1.05 \pm 0.05		1.09 \pm 0.04	1.12 \pm 0.04
		N.S.		N.S.
Collagen HO-proline (mg/dl)	0.15 \pm 0.05		0.98 \pm 0.15	0.12 \pm 0.05
free proline (mg/dl)	2.97 \pm 0.16		3.51 \pm 0.22	2.89 \pm 0.16
		$P = 0.074$		$P < 0.05$
GAG ^{a,b} (mg/dl)	2.37 \pm 0.05		2.50 \pm 0.03	2.17 \pm 0.05
		$P < 0.05$		$P < 0.001$

^a GAG, glycosaminogluconides.

^b Assuming that glucuronic acid is a third of the molecular weight of GAG.

weeks postpartum. However, there was a significant increase of plasma-free proline concentrations on the fourth postpartum day.

Another component of connective tissue are GAG. To prevent fortuitous increase of plasma concentrations of GAG by the disruption of leukocytes or platelets, after centrifugation a distinct layer of plasma was left undisturbed over the buffy coat. The plasma GAG concentrations were significantly increased during the fourth postpartum day.

Discussion. The concentration of plasma total protein-hydroxyproline in the nine pregnant women was the same as that in another group of 12 women during their third trimester (1.02 \pm 0.08 vs 1.05 \pm 0.11) and was not different from that of 15 normal healthy male volunteers (1.22 \pm 0.16) (unpublished observations). The plasma collagen-hydroxyproline was essentially zero also in the 15 normal volunteers as it was in 21 women during their third trimester and in nine at 6 weeks postpartum. These observations suggest that most, if not all, of the normal plasma protein-hydroxyproline is indeed accounted for by Clq. Nevertheless, changes of extraskelatal collagen turnover (hydrolysis) can give readily measurable increases of plasma protein-hydroxyproline, probably collagen, which is not due to changes to Clq concentrations.

The rise of plasma GAG during uterine

involution suggests that hydrolysis of GAG in extraskelatal connective tissue is associated with increased plasma concentrations of these compounds.

Summary. The information obtained in a prospective study of plasma concentrations of connective tissue components during the third trimester of pregnancy, 4 days, and again at 6 weeks postpartum, suggests that the increased concentrations of plasma protein-hydroxyproline (hypro-protein), free proline, and GAG are associated with increased rate of hydrolysis of extraskelatal connective tissue components.

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