A CHROMATOGRAPHIC STUDY OF HISTIDINURIA OF PREGNANCY

by

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Pregnancy histidinuria was first demonstrated by Voge (1929) and since then the phenomenon has been confirmed by a number of investigators, (Racker, 1940; Langley, 1941 and Page 1943). More recently, Wallraff *et al,* (1950) studied the excretion of aminoacids in pregnancy and reported a significant increase in histidine excretion in normal pregnancy.

Two theories have been advanced to explain this phenomenon. Boxer and Kapellar-Adler (1947) suggested that the gonadotrophic hormones inhibited the activity of liver histidase and thus increased the excretion of histidine due to its reduced catabolism. Chattaway (1947) studied histidine excretion during the normal menstrual cycle with a view to correlate the histidine excretion with gonadotrophic activity. He, however, could not find any such correlation. Shinde *et al* (1965) on the contrary, reported increased liver histidase activity in pregnant rats.

The other theory explained pregnancy histidinuria as a consequence of renal changes associated with pregnancy. Page *et al* (1954) showed by renal clearance studies that increased histidine excretion in normal pregnancy was largely due to associated renal changes like increased glomerular filtration rate, increased tubular blood flow, reduced tubular reabsorption, etc. This explanation got further evidence from observations of Lawrie (1947) and Wallraff *et al* (1950) who showed increased excretion of tyrosine and a number of other aminoacids.

We have tried to re-examine this phenomenon by studying histidine excretion in the different trimesters of pregnancy in order to ascertain any definite pattern of excretion. Serum histidine estimations were also done simultaneously to find out the possibility of any correlation between serum level and excretion.

Material and Methods

Serum and urinary histidine level was determined in 25 normal, healthy, non-pregnant females consisting mostly of medical students. After establishing the normal level, histidine determinations in serum and urine were done in 25 pregnant cases. These included 5 cases of first trimester, 6 of second, 7 of third, 4 of toxaemia associated with pregnancy and 3 postpartum cases. All these cases were

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drawn from the hospital attached to the college.

Fasting blood was drawn, allowed to clot and serum separated. 1 ml of serum was deproteinized and the aqueous extract so obtained was used for histidine determination. 24 hour urine was collected under toluene, its volume measured and a suitable aliquot used directly.

Histidine in urine and the aqueous extract of serum was separated by circular paper chromatography on Whatman No. 1 Paper using n-butanol: acetic acid: water (4:1:1.6) as the solvent. The chromatograms were run overnight, dried at room temperature and sprayed with Pauly's sulphanilic acid reagent to locate the histidine spots (Fig. 1). These spots were cut out and eluted in 2.5 ml. of distilled water at 50°C. The colour density of the eluted solution was measured at 420 mu. The results were calculated to give histidine concentration in mg per 100 ml. of serum and per 24 hour urine.

Results

24 hour excretion of histidine in non-pregnant females ranged from 77.27 mg. to 285.51 mg with a mean of 146.99 mg. In the first trimester of pregnancy, the mean excretion was 208.92 mg. with a range of 126.50 to 366.66. The excretion increased further in the second trimester when the mean excretion was 281.71 mg with a range of 156.37 to 383.50 mg. The third trimester period showed a sudden, sharp increase, recording a mean excretion of 419.60 mg and a range of 244.84 to 519.29 mg. There is, obviously, a gradual increase in histidine excretion with advance of pregnancy with peak excretion towards the end of gestation. Subjects of pregnancy toxaemia (Fig. 2) showed considerably reduced excretion of the order of 40.84 mg (Table I).

TABLE I

Histidine in Urine of Nonpregnant and Pregnant Females

Urine Histidine mg/24 hour	Nonpregnant Female	I Trimester	II Trimester	III Trimester	Toxaemia	After Delivery
Mean	146.49	208.92	281.71	419.60	40.84	184.01
Range	77.27-285.51	126.5-366.66	156.37-383.50	244.84-519.21	0-72.11	130.34-215.95
S.D.	51.93	93.10	97.44	110.09	33.76	46.53
S.E.	10.38	41.64	39.78	41.56	16.88	26.89

't' value:—(Students 't' test)

Between normal and II Trimester	— 3.2
Between Normal and III Trimester	— 6.3
Between Normal and Toxaemia	— 5.33
Between I Trimester and III Trimester	— 3.5
Between II Trimester and III Trimester	— 2.4

Excretion of histidine returned towards normal levels after delivery.

Serum histidine concentration did not show any significant change throughout the course of pregnancy. In toxaemia cases, however, the concentration was significantly more than the normal (Table II).

TABLE II

Histidine in Serum of Nonpregnant and Pregnant Females

Serum Histidine mg. per cent	Non-pregnant Female	I Trimester	II Trimester	III Trimester	Toxaemia
Mean	2.45	2.51	2.205	2.993	3.457
Range	0.85-4.38	1.81-3.75	1.13-3.00	1.27-4.09	2.97-4.46
S.D.	0.81	1.025	0.82	1.099	0.608
S.E.	0.16	0.45	0.41	0.882	0.304

't' values:— (Students 't' test)
Between Normal and Toxaemia —2.9

Discussion

The present study clearly establishes the phenomenon of pregnancy histidinuria. There are, however, certain variations in our observations and those reported earlier. Chattaway (1947) reported peak histidine excretion in the 5th month of pregnancy. Similarly, Ruttinger *et al* (1954) observed maximum excretion in the 4th month. We have observed a gradual increase in histidine excretion during the first 6 months and then a sudden sharp increase in the third trimester. Most of our third trimester cases were in the 8th or 9th month of pregnancy, indicating peak histidine excretion towards the end of gestation. Shinde and Agarwal (1968) had reported a similar observation in pregnant rats.

The study also confirms considerably lowered excretion of histidine in toxaemia as was reported by Langley (1941). Similarly, the aminoacid excretion drops to almost normal level after delivery. In 2 subjects, third trimester histidine excretion was 519.21 mg and 513.33 mg and this came down to 206.35 mg and 215.35 mg respectively on 7th and 20th day after delivery. Similar results have been reported by Ruttinger *et al* (1954). This sudden lowering of the aminoacid excretion after delivery has been explained to be due to a greater demand during lactation.

The concept of gonadotrophic inhibition of liver histidase as a cause of increased

histidine excretion during pregnancy was advocated by Boxer and Kapeller-Adler (1947). A number of investigators have attempted to verify this mechanism. Page (1943) and Edelbacher and Heitz (1946) could not substantiate the hormonal effect. Similarly, Chattaway (1947) in his studies on histidine excretion during normal menstrual cycle, failed to obtain any correlation between excretion and hormonal activity. If chorionic gonadotrophic hormones inhibit liver histidase, histidine excretion should be expected to be maximum during the first trimester of pregnancy when these hormones are secreted at a high concentration. This has not been the case in this study. Also, Shinde *et al* (1966) failed to observe any inhibitory effect of oestrogen, progesterone and cortisone on liver histidase in pregnant rats. In fact, they noted an increased enzyme activity.

The derangement in enzyme activity might be expected to have some effect on serum level of the amino acid also but this too does not happen. The serum level of histidine remains within normal limits throughout pregnancy and has no correlation with urinary excretion. Page (1954) had observed a high rate of histidine excretion even at normal serum level. He had further noted that oral administration of histidine resulted in a slow rise of blood concentration in pregnancy. He concluded that pregnancy is associated with a slow rate of histidine transfer across both the intestinal and renal tubular barrier.

Our observations of a gradual rise in histidine excretion during pregnancy and unchanged serum concentration does not justify the enzyme inhibition mechanism but points firmly to the renal origin.

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See Figs. on Art Paper II