A STUDY OF VARIATIONS IN PLASMA AMINOACID LEVELS IN DIFFERENT PHASES OF THE MENSTRUAL CYCLE

by

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Soupart (1960, 62) reported general lowering of the plasma concentration of all individual aminoacids in the secretory phase of the menstrual cycle. Some amino acids were affected more than others. Craft and Wise (1969a) related this to the release of progestrone following ovulation. The work of Landau and Lugibihl (1961) supports this view. These workers gave 50 mg of progesterone intramuscularly daily to men and women and there was an increase in urinary nitrogen due mainly to urea. There were no changes in amino acid excretion. Similarly, the results of oral and intravenous glycine tolerance tests suggested that there was increased utilization of amino acids in the second half of the menstrual cycle (Craft and Wise, 1969b). Although oestrogen production was also raised in the ovulatory phase it has not been considered significant in relation to plasma nitrogen changes observed in the ovulatory phase (Craft and Wise, 1969b). The total plasma or free amino acid values showed no change after administration of diethylstilboestrol (Zinneman *et al* 1965).

Release of endogenous progesterone has again been considered as the most important factor in lowering the fasting plasma amino acid levels in early pregnancy (Bonsnes, 1947).

Could the lowering of plasma amino acids in early pregnancy be taken as an index of progesterone production? Evidently the answer to the question is being sought expecting probable utility of the investigation in diagnosing threatened abortion. But the complete analysis of individual plasma amino acids as done by Soupart (1959, 1960) will be too laborious for the purpose. Estimation of total plasma amino acids by a chemical method may not be very helpful as all amino acids are not affected to the same degree (Soupart 1960). In a simple unidimensional chromatographic method (Saini, 1971), five groups of amino acids are obtained. In this investigation an attempt is made to find out which group is maximally affected with variation in endogenous progesterone release. For the purpose twenty females have been studied in the post menstrual and the luteal phases.

Material and Methods

Twenty female medical students bet-

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Received for publication on 26-10-71.

ween the age of seventeen and ninteen years, having regular menstrual cycles, were selected. Fasting blood samples were taken in heparinized sampling tubes. Two samples between 2-7 days and 20-25 days of the cycle were taken between 8-10 A.M.

Plasma amino acids were separated by unidimensional chromatography as described by Saini (1971). 350 μl of plasma, 0.8 ml ethyl alcohol and 50 μl of distilled water were mixed in a tube 6.0 $\times$ 0.8 cm and shaken vigorously. The whole mixture was applied as a rectangular spot (5.0 $\times$ 1.0 cm) on Whatman no. 3 MM filter paper (28 $\times$ 22 cm) depositing half the volume on one side of the paper and half on the opposite side. The solvent used was n-butyl alcohol-acetic acid-water (12:3:5). Three consective runs (1 h, 4 h, 16 h) were given. The paper was stained with 0.2% ninhydrine solution and dried. For full development of the colour of the bands, the paper was kept in an oven at a temperature of 95°-100°C for 5 minutes. The chromatogram was then stained with alcoholic copper nitrate. The colour of the bands changed from purple to salmon red. The bands were cut out into small strips and eluted in 8 ml of methanol. After one hour the density of the colour was measured at 509 mu in a spectrophotometer.

The separation of amino acids as observed in the unidimensional chromatograms obtained in the present study is illustrated in Fig. 1. It is seen that the amino acids separate out into a number of groups out of which those listed in Table 1 have been used for quantitation. Each group has been quantitated in terms of one amino acid as indicated in Table 1.

Standard solution of various strengths of the amino acids leucine, valine, alanine, threonin and glycine were applied on filter paper and treated in the same manner as the samples. Standard curves were plotted from the data obtained and used to quantitate the spots in chromatograms. Results were expressed as μg/ml

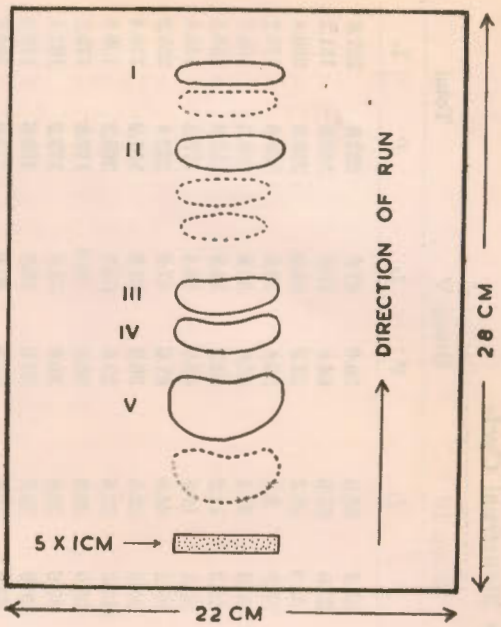

Fig. 1

TABLE I

Amino acid Groups Separated from Human Plasma by Unidimensional Chromatography

Group	Amino acids	Amino acids in terms of which spot is quantitated
I	Leucine Isoleucine	Leucine
II	Valine Methionine	Valine
III	Alanine	Alanine
IV	Threonin Glutamic acid	Threonin
V	Glycine Serine Glutamine Taurine	Glycine

TABLE II

Plasma Amino acid Levels (μg/ml) in 20 Subjects During Post-menstrual (P) and Leuteal Phases (L) of the Menstrual Cycle

	Group I		Group II		Group III		Group IV		Group V		Total	
	P	L	P	L	P	L	P	L	P	L	P	L
1	42.0	42.0	47.6	47.6	78.4	61.6	58.8	58.8	56.0	47.6	282.8	257.6
2	42.0	36.4	44.8	30.8	56.0	28.0	33.6	61.6	64.4	30.8	240.8	151.2
3	44.8	44.8	50.4	50.4	47.6	47.6	42.0	25.2	53.2	56.0	238.0	260.4
4	56.0	28.0	67.2	28.0	56.0	30.8	39.2	8.4	50.4	28.0	268.8	123.2
5	44.8	36.4	58.8	42.0	56.0	36.4	33.6	22.4	56.0	30.8	249.2	168.0
6	33.6	22.4	42.0	19.6	44.8	25.2	39.2	11.2	53.2	30.8	212.8	109.2
7	33.6	30.8	36.4	30.8	42.0	30.8	25.2	19.6	42.0	36.4	179.2	148.4
8	36.4	42.0	47.6	47.6	61.6	53.2	39.2	44.8	47.6	47.6	232.4	235.2
9	28.0	28.0	33.6	30.8	33.6	36.4	25.2	22.4	30.8	30.8	151.2	148.4
10	33.6	30.8	47.6	42.0	42.0	39.2	117.6	33.6	22.4	53.2	263.2	198.8
11	33.6	36.4	33.6	36.4	44 8	47.6	28.0	28.0	30.8	30.8	170.8	179.2
12	28.0	30.8	36.4	33.6	36.4	30.8	47.6	33.6	30.8	33.6	179.2	162.4
13	30.8	30.8	28.0	33.6	42.0	56.0	30.8	25.2	28.0	28.0	159.6	173.6
14	33.6	25.2	36.4	33.6	47.6	33.6	33.6	19.6	22.4	42.0	173.6	154.0
15	33.6	30.8	36.4	33.6	39.2	39.2	22.4	19.6	58.8	47.6	190.4	170.8
16	44.8	30.8	53.2	33.6	28.0	16.8	47.6	33.6	64.4	50.4	238.0	165.2
17	36.4	28.0	44.8	25.2	50.4	25.2	22.4	19.6	58.8	58.8	212.2	156.8
18	42.0	33.6	47.6	33.6	61.6	47.6	44.8	30.8	42.0	30.8	238.0	176.4
19	39.2	39.2	42.0	42.0	42.0	39.2	25.2	33.6	28.0	28.0	176.4	182.0
20	28.0	33.6	28.0	33.6	42.0	28.0	36.4	33.6	33.6	33.6	168.0	162.4

TABLE III

Mean Plasma Amino acid Levels in Various Phases of Menstrual Cycle

Amino acids	Post menstrual		Leuteal		't' test
	Means	S.E.	Means	S.E.	
Group I	37.28	1.58	33.04	0.89	P <0.05
Group II	43.12	2.21	35.42	1.72	P <0.01
Group III	47.75	2.55	37.66	2.58	P < .001
Group IV	39.62	4.60	29.26	4.70	P <0.1
Group V	43.68	3.17	38.78	2.32	P <0.1
Total of above groups	211.26	8.96	174.15	8.70	P <0.05

plasma and were analysed using paired 't' tests.

Observations

The levels of plasma amino acids in the postmenstrual and leuteal phases of the menstrual cycle observed in various groups of amino acids are given in Table II. The results are analysed in Table III.

It is seen that in each case the mean value is greater in the postmenstrual phase. The differences are significant for amino acid group I, II & III and also for the total of all groups.

Discussion

Craft and Wise (1969a) reported significant lowering of the plasma a-amino nitrogen level in the secretory phase of the menstrual cycle as compared to the postmenstrual phase. The results of the present investigation are in agreement with their findings, as there is significant lowering of the total amino acid level in the luteal phase as compared to the postmenstrual phase (Table III). Soupart (1960, 1962) also reported a general lowering of the plasma concentrations of all individual amino acids in the secretory phase, the lowering being statistically significant for alanine, lysine, proline, serine and threonine. Considering the individual groups (Table III) of present series, there is lowering in the concentrations of the all groups except group V (Glycine, serine, glutamine and taurine) in the luteal phase as compared to the postmenstrual phase. The most significant differences have been obtained for group III, comprised by alanine alone. As these differences in amino acids levels have been related to the changes in levels of endogenous progesterone (see under introduction), plasma alanine levels (using the present method of investigation), appear to be most faithfully related to the endogenous progesterone levels.

Determination of plasma alanine levels by the simple method used in this investigation may therefore provide a convenient index of plasma progesterone levels. Using the same technique it will be interesting to study serial plasma samples from women in early pregnancy. With advance in pregnancy, the level of endogenous progesterone goes on increasing. The levels of the various groups of amino acids (especially those of alanine), separated in the present technique should go on decreasing in concentration.

Summary

Plasma amino acids have been studied by unidimensional paper chromatography in the postmenstrual and luteal phases in twenty adult women. Amino acid levels are distinctly higher in the postmenstrual phase, than in the luteal phase. The differences are statistically significant.

References

1. Bonsnes, R. W.: J. Biol. Chem. **168**: 345, 1947.
2. Craft, I. L. and Wise, I. J.: Nature. **222**: 487, 1969. a
3. Craft b, I. L. and Wise, I. J.: J. Obst. & Gynec. Brit. Cwlth. **76**: 928, 1969.. b
4. Landau, R. L. and Laugibil, K.: Rec. Progr. Hormone. Res. **17**: 249, 1961.
5. Saini, A. S.: Clin. Chim. Acta. **31**: 479, 1971.
6. Soupart, P.: Clin. Chim. Acta. **5**: 235, 1960.
7. Soupart, P.: Clin. Chim. Acta. **4**: 265, 1959.
8. Soupart, P.: Acta. Clin. Belg. **9**: 297, 1954.
9. Soupart, P.: Amino Acid Pools. Edited by Holden. J. T. Elsevier, Amsterdam. 227, 1962.
10. Zinneman, H. H., Musa, B. U. and Doe, R. P.: Metabolism. **14**: 1214, 1965.