"STUDY OF ALKALINE PHOSPHASTASE ACTIVITY IN NORMAL PREGNANCY"

by

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Several iso-enzymes of orthophosphoric monoester phosphohydrolase, better known as alkaline phosphatase have been distinguished by the application of electrophoretic, chromatographic, and immunochemical procedures to sera and extracts prepared from different tissues specially bone, liver, kidney and placenta. Recent work has shown that the progressive rise in serum alkaline phosphatase activity during pregnancy is due to an enzyme which resembles the placental enzyme in its physico-chemical properties like thermal stability, electrophoretic, and immunochemical characteristics (Neale and co-workers, 1964; McMaster and co-workers, 1964). Alkaline phosphatase is also well known for being relatively non-specific in its substrate requirements and several substrates have been proposed for the determination of its activity (Wilkinson, 1965). As regards thermal stability, it has been well established that placental alkaline phosphatase is the only known human alkaline phosphatase stable on heating for half an hour at 56°C (Neale and co-workers, 1965). Recently, the possibility of the changes in the level of this enzyme activity during pregnancy as an aid to placental function had been advocated by Lavine and co-workers (1965, 1966) and Curzen and Morris, (1968). In addition p-nitrophenyl phosphate has been lately chosen for the automatisation technique as the substrate of choice. However, it does not seem permissible to use a factor to convert units of one method into units of the other method (Tietz *et al* 1967). In the present investigation a comparative data of the total, and heat stable enzyme activities at different stages of gestation, labour and early puerperium, cord blood, and of placenta with reference to the three well known substrates, namely, B-glycerophosphate, phenylphosphate and p-nitrophenyl phosphate is presented.

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Material and Methods

A total of 222 samples of sera were analysed from the following groups of cases:

(a) Thirty non-pregnant healthy adult women between 18-28 years of age.

(b) Thirty women each in the three different trimesters of pregnancy.

(c) Forty-four women in labour between 36-40 weeks of pregnancy.

(d) Thirty women in the puerperium between four to seven days after delivery.

(e) Thirty-eight cord blood samples from babies delivered at 36-40 weeks' of gestation.

(f) Thirty placentae collected at the time of delivery from the above subjects.

All subjects were healthy pregnant women with no detectable complications of pregnancy and labour. Samples of blood were obtained after overnight fast in all cases except those in labour. Sera were separated and stored at 4°C and all estimations completed within a week. In each sample, total and heat stable alkaline phosphatases were determined with the three substrates, namely — glycerophosphate (Bodansky, 1933); Phenyl phosphate (King, 1965) and p-nitrophenyl phosphate (Bessey *et al* 1946). Ten per cent placental homogenates were prepared in distilled water after thorough washings with ice cold normal saline and used as the enzyme source after appropriate dilution. Corrections were made for the residual blood in the homogenate (Mc. Master *et al* 1964).

For estimation of heat stable enzyme activity, aliquots of sera were heated at 56°C for 30 minutes, quickly brought to room temperature and then the enzyme activity was determined using different substrates. The heat labile enzyme activity was obtained by subtracting the activity of the heat stable enzyme from that of the total enzyme in each instance.

The enzyme activity with respect to each substrate has been expressed as milli international unit (M.I.U.) instead of the conventional Bodansky, King Armstrong and Bessey-Lowry units — so that the comparison could be made on a uniform basis. One milli international unit is the micromole substrate decomposed per minute per 100 ml. in the case of sera and per gram of wet tissue in the case of placenta.

Results

The total enzyme activity in relation to the three substrates used in the different groups studied is shown in Table 1. Progressive increase in the enzyme activity during pregnancy is noted with all the three substrates with highest levels during labour. A significant rise could be detect-

TABLE I

Serum total alkaline phosphatase activity (m.I.U) three substrates

Subjects	No. of cases.	Substrate		
		-glycero phosphate	Phenyl phosphate	p- nitro-phenyl phosphate
Adult women (non-pregnant)	30	12.47(±3.78)	42.20(±12.2)	22.88(±4.84)
First trimester (6-14) weeks	30	16.60 (±7.07)	49.91 (±18.0)	22.38 (±6.85)
Second trimester (15-30) weeks.	30	19.17 (±1.24)*	62.69 (±19.59)*	34.40 (±14.36)
Third trimester (31-40) weeks	30	32.24 (±12.96)*	104.37 (±43.31)*	40.91 (±21.21)*
Labour	44	49.74 (±21.6)*	158.76 (±74.83)*	62.29 (±24.88)*
Post-partum (4-7) days	30	31.32 (±15.55)*	98.90 (±41.39)*	36.57 (±20.71)*
Cord-blood	28	34.32 (±15.23)*	121.98 (±58.9)*	54.94 (±38.08)*

+ Figures in brackets indicate the standard deviation of the mean activity.
* Significant at 1% level as compared to non-pregnant level.

ed from the second trimester onwards ($p < 0.01$). The total enzyme activity in cord blood was also significantly raised ($p < 0.01$) as compared to adult non-pregnant controls. The post-partum levels though showing a reduction from the third trimester levels, were markedly above the non-pregnant levels ($p < 0.01$).

Table II shows the heat stable alkaline phosphatase activity in serum and placental homogenates in relation to the three substrates in the same group of subjects. Significant increase from the second trimester with all the three substrates could be detected. If these activities were expressed in the conventional units (B.U., K.A.U. and B.L.U.) only with p-nitrophenyl phosphatase, a significant rise could be demonstrated in the second trimester. Post-partum (4-7 days) level of the heat stable enzyme activity in relation to the three substrates was also significantly higher than those of non-pregnant controls. The cord blood enzyme activity was similar to that of the non-pregnant women.

The heat labile enzyme activity, as shown in Table III was significantly rais-

TABLE II

Serum and placental heat stable alkaline phosphatase activity (m.I.U.) with three substrates

Subjects	No. of cases.	Substrate		
		β-glycero phosphate	phenyl phosphate	p- nitro-phenyl-phosphate.
Adult-women (non-pregnant)	30	2.70 (±1.45)	9.37 (±6.75)	7.01 (±4.84)
I trimester (6-14) weeks	30	3.78 (±3.13)	9.16 (±7.81)	6.01 (±4.32)
II Trimester (15-30 weeks)	30	6.86 ± (6.48)*	19.45 (±20.59)*	15.87 (±9.69)*
III trimester (31-40) weeks	30	18.36 (± 11.88)*	52.9 (± 33.51)*	27.11 (±3.67)*
Labour	44	30.24 (±14.74)*	87.12 (±40.47)*	40.24 (± 18.04)*
Post-partum (4-7) days	30	10.53 (±8.96)*	27.90 (±26.48)*	14.53 (±12.19)*
Cord blood	28	1.94 (±0.59)	9.23 (±5.00)	6.513 (±12.19)
Placenta	30	4.45 (±2.96)	10.44 (±6.42)	6.61 (±2.61)

+ Figures in brackets indicate the standard deviation of the mean activity.
* Significant at 1% level as compared to non-pregnant level.

TABLE III

Serum Heat Labile Alkaline Phosphatase Activity with the Three Substrates

Subjects	No. of cases.	Substrote		
		-glycero phosphate	phenyl phosphate	p-nitro-phenyl phosphate.
Adult women (non-pregnant)	30	9.72 (±3.67)+	32.94 (±9.66)	15.87 (±3.84)
First trimester (6-14) weeks	30	12.28 (±7.00)	40.26 (±15.55)	18.04 (±11.69)
Second (trimester (15-30) weeks.	30	12.42 (±7.56)	43.31 (±11.01)*	18.54 (±7.51)
Third trimester (31.40) weeks.	30	12.85 (±6.32)	51.40 (±22.37)*	16.03 (±7.85)
Labour	44	19.22 (±10.58)*	71.50 (±43.31)*	21.88 (±11.69)*
Post-partum	30	20.36 (±10.80)*	66.95 (± 36.14)*	22.55 (±13.36)*
Cord blood	28	32.94 (±14.90)	112.61 (±43.52)*	40.75 (±3.34)*

+ Figures in brackets indicate the standard deviation of the mean activity.
* Significant at 1% level as compared to nou-pregnant level.

ed with all the three substrates during labour, post-partum (4-7 days) and in cord blood. During pregnancy a significant rise in heat labile enzyme activity could however be demonstrated from the second trimester with phenylphosphate and not the other substrates.

Discussion

In the groups of women studied, the total and heat stable alkaline phosphatase activity with the three different substrates in the three trimesters of pregnancy were in agreement with results reported by previous workers (McMaster and co-workers, 1964; Sadovsky and Zuckerman, 1965). The increase in the total enzyme activity along with the heat stable component was progressive upto labour. However, there is no general agreement about the duration of pregnancy at which alkaline phosphatase starts rising. Bodansky (1963) observed the rise to begin in the fourth month whereas Meranze and co-workers (1937) noted a significant rise not earlier than the seventh month. The rise of enzyme activity above normal levels was reported from the fifth month by Zuckerman and co-workers (1965) and Curzen, (1966), though statistically significant rise has been observed only in the third trimester by most workers. In the present series, no significant rise occurred with the substrates in either the total or HSAP in the first trimester. However, a significant rise of the heat stable enzyme activity with all the three substrates, was observed from the second trimester onwards. The wide range of variability particularly with reference to the heat stable fraction observed was also reported by several other workers (Kitchener and co-workers, 1965; Beck and Clark, 1950; Bodansky, 1939 and Meranze and co-workers, 1937). These variations have been attributed to several factors like changes in plasma volume, placental mass and permeability, rate of enzyme synthesis and clearance, body surface area, sex of foetus and mixture of isoenzymes. Because of this variability, AP and HSAP determinations have been considered unsuitable for use as placental function tests (Watson and co-workers, 1965) or if used, the estimations have to be done daily (Curzen and Morris, 1968). The transient rise in total and HSAP activity noted during labour with all the three substrates has been reported by other workers as well (Meade and Rosalki, 1963) and could be attributed to admixture of blood from the chorio-decidual space, though this hypothesis has to be substantiated by further work. By the seventh day after delivery, the levels had not reached the non-pregnant levels and as reported by Sadovsky and Zuckerman, (1965) this may take about 20 days. The same authors reported on total enzyme activities in B.L. Units throughout pregnancy and commented that the increment in pregnancy with p-nitrophenyl phosphate (B.L. Units) was considerably less than that with ß-glycerophosphate (B.U.) With placental extracts also the same was observed. Cord blood values for total enzyme activity were higher than in non-pregnant controls but were similar to maternal serum during labour with an important qualitative difference in that 90% or more of the activity was due to the heat labile fraction. These observations are in agreement with those of (Meade and Rosalki, 1963; Speert and co-workers, 1959; McMaster and co-workers, 1950; McMaster and co-workers, 1964 and Sadovsky and Zuckerman, 1965).

Hunter, (1969) and Curzen and Southcombe (1970) suggested that the placental A.P. observed during pregnancy is undoubtedly heat stable but suggested a

critical temperature of 65°C and found that this method also reduces the scatter considerably.

The study of isoenzymes of alkaline phosphatase has become more complex by reports of further heterogenity related to genetic polymorphism and the relationship of several variables like racial factors, blood groups, etc. (Boyer, 1961, Arfors and co-workers, 1963; Robinson, and Goldsmith, 1967).

In the present study, increasing order of the activity of alkaline phosphatase with respect to the three substrates was observed as -Phenyl phosphate, para nitrophenyl phosphate, ß-glycerophosphate. Certain features with reference to the heat labile enzyme also need comment. A significant increase in heat labile enzyme during the second and third trimesters was observed with phenyl phosphate but not with the other two substrates. No explanation can be offered at this stage for this observation.

Summary

Total and heat stable alkaline phosphatase activities in serum with reference to glycerophosphate, phenyl phosphate and p-nitrophenyl phosphate as substrates were determined in healthy pregnant women at various stages of gestation, during labour and early puerperium, in cord blood and placenta.

Progressive increase during pregnancy in total and heat stable alkaline phosphatase levels was noted with all the three substrates, maximum levels being observed during labour. Statistically significant rise in the heat stable fraction could be detected in the second trimester.

The cord blood showed significantly higher total and heat labile activities with all the three substrates, when compared to non-pregnant controls.

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