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Influence of Menstrual Cycle on Serum Cholinesterase

ANNE FAIRBROTHER,* SHELDON L. WAGNER,† SHIRLEY WELCH,‡ AND BRADFORD B. SMITH§

*U.S. EPA Environmental Research Laboratory, Corvallis, Oregon 97333; †Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331; ‡Medlab, Inc., Portland, Oregon 97208-3380; §College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97333

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This study examined whether the variability in cholinesterase (ChE) values among and within women may be attributed to phase of menstrual cycle and/or circulating progesterone concentration. Blood was drawn from 21 female subjects once a week for 8 weeks and analyzed for ChE activity and for progesterone concentration. Women ranged in age from 25 to 55 years and five used exogenous hormones (oral contraceptives, estrogen supplements, or progesterone therapy); one woman became pregnant during the study. There was a significant positive correlation between serum ChE and progesterone values only for the two women on oral contraceptives although there were large weekly variations within individuals (CV: 4–32%). Age significantly affected ChE values with 36–40 year olds having the lowest values and 30–35 year olds the highest. This variation in serum ChE probably is due to the influence of some sex steroid but, in women, there is not a direct one-to-one relationship between the enzyme and progesterone. However, when interpreting ChE tests used to monitor exposure of women to pesticides age and hormone intake must be considered in order to avoid false positive results.

INTRODUCTION

Cholinesterase (ChE) measurements are performed routinely on agricultural workers at risk of exposure to organophosphate and carbamate pesticides (Duncan *et al.*, 1986; Holmes, 1965; Willis, 1972). However, interpretation of results from these enzyme assays is confounded by the variability of the normal, circulating levels of the enzyme. Variation of plasma ChE values among normal individuals exceeds 200% (see review by Augustinsson, 1955). Additionally, there are daily variations within single individuals of up to 13% in red blood cell (RBC) ChE and up to 23% in serum ChE (Augustinsson, 1955; Fryer *et al.*, 1956).

The inherent variability of plasma and RBC ChEs may be caused by fluctuations in the concentrations of sex steroids thought to exhibit some influence, if not direct control, on ChE production (Illsley and LaMartinier, 1981). Sexual maturation in females affects plasma cholinesterase, with values significantly lower in menarchial women than in women of premenstrual or postmenstrual status (Lepage *et al.*, 1985). Oral contraceptive medication has been shown to decrease plasma and RBC ChE (Robertson, 1967; Sidell and Kaminskis, 1975). ChE levels are decreased during pregnancy and immediately postpartum. In the third trimester ChE values are decreased up to 25% compared with those of nonpregnant women (Whittaker, 1980). These observations led us to hypothesize that ChE values may vary in females during the menstrual cycle in response to changes in

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circulating hormones. The objective of this study was to determine if the large variability in ChE values among and within women may be attributed to phase of menstrual cycle and/or circulating progesterone concentration.

MATERIALS AND METHODS

Samples. Samples of whole blood were drawn into 10-ml vacutainer clot tubes from the cubital veins of 21 volunteer female subjects between 0830 and 0930 hr once a week for 8 weeks. Subjects abstained from eating for at least 8 hr prior to each sampling period. Blood was allowed to clot at room temperature (approximately 30 min) and centrifuged for 10 min at 2,000*g*, and the serum was separated from the cells and divided into two polypropylene screw cap vials. One vial from each subject was frozen at −75°C for up to 8 weeks prior to analysis for progesterone. The other aliquot was analyzed immediately for serum ChE, γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Hepatic enzyme screens were conducted to detect women with liver malfunction resulting in abnormally low ChE values. An additional 5 ml of blood was collected from five subjects into vacutainer tubes containing sodium heparin. These were kept in the dark at room temperature for up to 24 hr until analyzed for plasma and RBC ChEs. Urine samples were obtained from all subjects immediately following blood collection. Samples from Weeks 3 and 4 were sent to the Oregon Department of Agriculture Diagnostic Testing Laboratory (Salem, OR) for analysis of organophosphate parent compounds and metabolites to detect exposure to ChE inhibitors that might confound the results of the study. The remaining urine samples were stored at −20°C until the termination of the experiment in the event that an unusually low blood ChE value was obtained. Records were kept of each subject's menstrual cycle, reproductive history, birth control method, medications, possible exposure to ChE-inhibiting insecticides, and history of liver disease.

Analytical methods. Total serum ChE activity was determined by a modification of the Ellman *et al.* (1961) technique using a kit produced by Ciba–Corning Diagnostics (Oberlin, OH) and a Gilford SBA-300 autoanalyzer. Final concentrations (per liter) of reagents in the test were 4.0 mmol of proprionylthiocholine as substrate and 0.25 mmol of 5,5-dithio-bis-(2)nitrobenzoic acid in pH 6.8 phosphate buffer. Assays were conducted at 30°C and at a wavelength of 405 nm. Absorbance readings were taken six times in 15 sec beginning 22 sec after start of the reaction. Reference standards were included with each day's run. All samples were run in duplicate; assays were repeated if duplicate runs differed by more than 10%. Results of the two runs were averaged for purposes of analyses. Results were expressed as IU/liter.

Plasma and RBC ChEs were determined electrometrically with a modification of the method of Michel (Henry *et al.,* 1974). ChE activity was determined by measuring the drop in pH after incubation of specimen (lysed red cells or plasma) with acetylcholine chloride for 1 hr at 25°C. Correction was made for the drop in pH resulting from nonenzymatic hydrolysis of substrate during the incubation period. All specimens were run in duplicate and quality control samples were

included in each run. Duplicates were required to be within ±0.05 pH units. Results were expressed in pH units/hour.

GGT, AST, and ALT analyses were conducted following the methods of Demetriou *et al.* (1974), the International Federation of Clinical Chemistry (1975), and Henry (1968), respectively, using kits produced by Ciba–Corning Diagnostics. Values used for lag and read times and reaction temperatures were those suggested by Gilford for use with the SBA-300 autoanalyzer.

Serum progesterone concentrations were determined using a radioimmunoassay kit purchased from Diagnostic Products Corporation (Los Angeles, CA). The kit had been validated and licensed for measurement of progesterone concentrations in human serum. Samples were run in duplicate, none of which differed by more than 10%. All samples were determined in a single assay with an intra-assay coefficient of variation of 9.8, 8.5, and 8.0% for human serum internal standards with mean concentrations of 17.7, 3.5, and 1.2 ng/ml progesterone, respectively.

Urine samples were tested for the following organophosphates (EPA, 1980): diazinon, malathion, ethyl parathion, methyl parathion, fonofos, phorate, ethion, carbophenothion, methidathion, endosulfan, and acephate. Samples also were analyzed for the parathion metabolite *p*-nitrophenol. Minimum detectable levels were 0.02 ppm for the parent compounds and 0.08 ppm for the *p*-nitrophenol.

Statistics. All analyses were conducted using the SAS package for personal computers (SAS Institute, Inc., 1985). Effects of age and menstrual cycle on ChE and progesterone values were analyzed using a repeated measures analysis of variance technique with unequal cell sizes followed by a Student–Newman–Keuls comparison between means. Square root transformations of progesterone and RBC ChE were done prior to analyses to normalize variances. All other factors were normally distributed and so were not transformed. Coefficients of variation were calculated as the standard deviation expressed as a percentage of the mean. Correlation analyses were done by the method of least squares on data standardized to mean = 0 and standard deviation = 1.

RESULTS

Ages of the 21 women in the study ranged from 25 to 55 years. Five women took exogenous hormones (two were on oral contraceptives, two had had ovariohysterectomies and were on supplemental estrogen, and one suffered from premenstrual syndrome and was prescribed 20 mg methyroxyprogesterone daily for 13 days beginning on the 14th day of each menstrual cycle). Additionally, one woman had had a hysterectomy, with ovaries left intact, so menstrual cycle was indeterminate although hormone production continued. Another woman became pregnant approximately 2 weeks into the study. None of the women had abnormal liver function enzyme values (GGT, AST, ALT) during the study, nor was any organophosphate parent compound or metabolite found in urine samples collected from Weeks 3 and 4.

Analysis of variance showed that age ($P = 0.0002$) significantly affected serum ChE. Results were the same for plasma ChE and RBC ChE measured electrometrically. Women aged 36–40 had lower ChE values than women in other age groups; women aged 30–35 had the highest values (Table 1).

TABLE 1
MEAN AND STANDARD ERRORS OF CHOLINESTERASE AND PROGESTERONE VALUES FOR WOMEN IN EACH AGE CLASS

Age class (years)	Number of subjects	Cholinesterase[a]			Progesterone[c]
		Serum	Plasma[b]	RBC[b]	
25–30	7	4326[d] (221)	0.59 (0.05)	0.60 (0.02)	2.14 (0.62)
31–35	6	5086 (174)	1.00 (0.07)	0.63 (0.01)	3.38 (1.01)
36–40	4	4121 (242)	—	—	5.59 (1.22)
41–45	4	4936 (259)	0.71 (0.03)	0.54 (0.01)	2.14 (0.80)

[a] Serum values expressed as IU/liter. Plasma and red blood cell (RBC) values expressed as pH units/hr.
[b] Number of subjects = 2, 2, and 1 for age groups 25–30, 31–35, and 41–45, respectively.
[c] Expressed as ng/ml.
[d] N = 8 observations per subject times number of subjects.

There was a significant positive correlation (r = 0.60; P = 0.006) between serum ChE and progesterone values only for the two women on oral contraceptives (Fig. 1A). There also was a significant negative correlation (r = 0.74; P = 0.03) between serum ChE and RBC ChE values in one of these two women (RBC ChE was not measured in the other woman) (Fig. 1B). There was no significant correlation ($P > 0.05$) between serum ChE, plasma ChE, or RBC ChE values and progesterone values for any other woman either on an individual basis or when the data were grouped by age class. The woman taking progesterone medication for 13 days prior to onset of mensis did not have a decrease in ChE values at this time (no medication $\bar{x}$ = 6142 SE = 580 vs medication $\bar{x}$ = 6836 SE = 1045).

Data analyzed within each age class showed significant differences in serum ChE values between weeks of the menstrual cycle within each woman ($0.007 < P < 0.02$). Weekly variations of ChE values within individuals as measured by their coefficients of variation varied as little as 4% and as much as 32%. However, there was no consistent pattern among the women in how their values varied from week to week nor did hormone treatment significantly affect the amount of variation within an individual (Fig. 2). RBC ChE values also exhibited significant differences due to week of menstrual cycle but plasma ChE (measured electrometrically) did not.

Variability in ChE among individuals within an age class was greatest in 25–30 year olds (CV = 31.5%) and least in 31–35 year olds (CV = 19.6%) (Table 2A). Variation of ChE within an individual was greatest in 36–40 year olds (mean CV = 17.4%) and least in 41–55 year olds (mean CV = 6.8%) (Table 2B). Generally, plasma ChE and RBC ChE percent CVs paralleled those of serum ChE but were lower (Table 2).

Serum ChE values measured colorimetrically were significantly correlated (r = 0.94, P = 0.0001) with plasma ChE values measured electrometrically (Fig. 3). The slope of the regression line was 1.1, suggesting that both methodologies were measuring the same amount of enzyme activity.

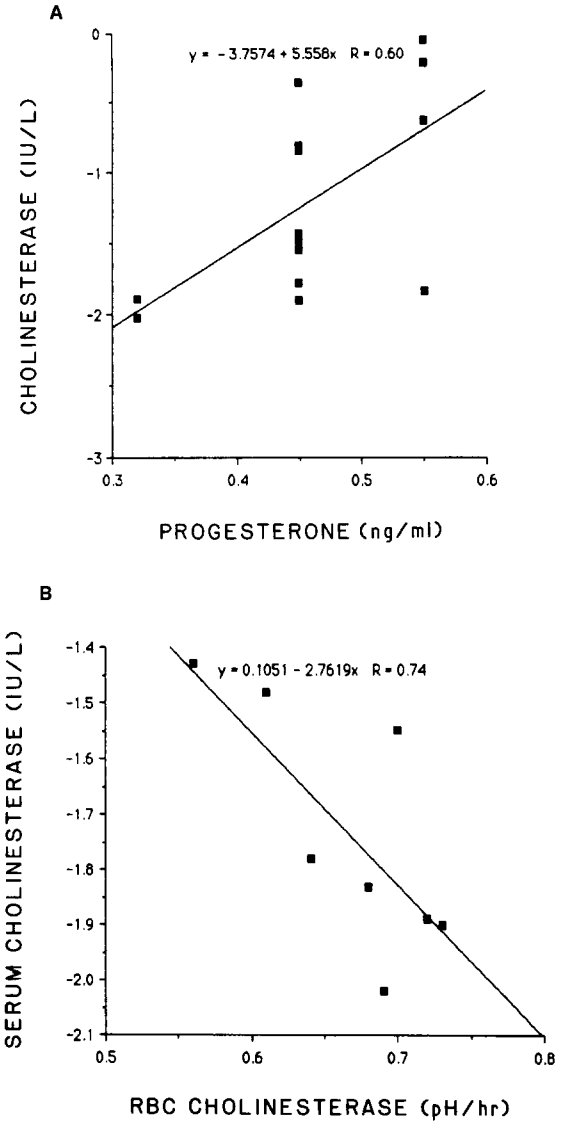

FIG. 1. (A) Serum cholinesterase values (IU/liter) as a function of progesterone (ng/ml) concentration in women taking oral contraceptives. (B) Correlation between serum and RBC cholinesterase (pH change/hr) activity in one woman taking oral contraceptives. Cholinesterase data standardized prior to analysis to the mean equal to zero and the standard deviation equal to one.

DISCUSSION

This study was designed primarily to examine the variability in serum ChE values within individual women. It was hypothesized that progesterone affects circulating levels of ChE since other studies have demonstrated a significant decrease in ChE in women taking oral contraceptives (Robertson, 1967; Sidell and

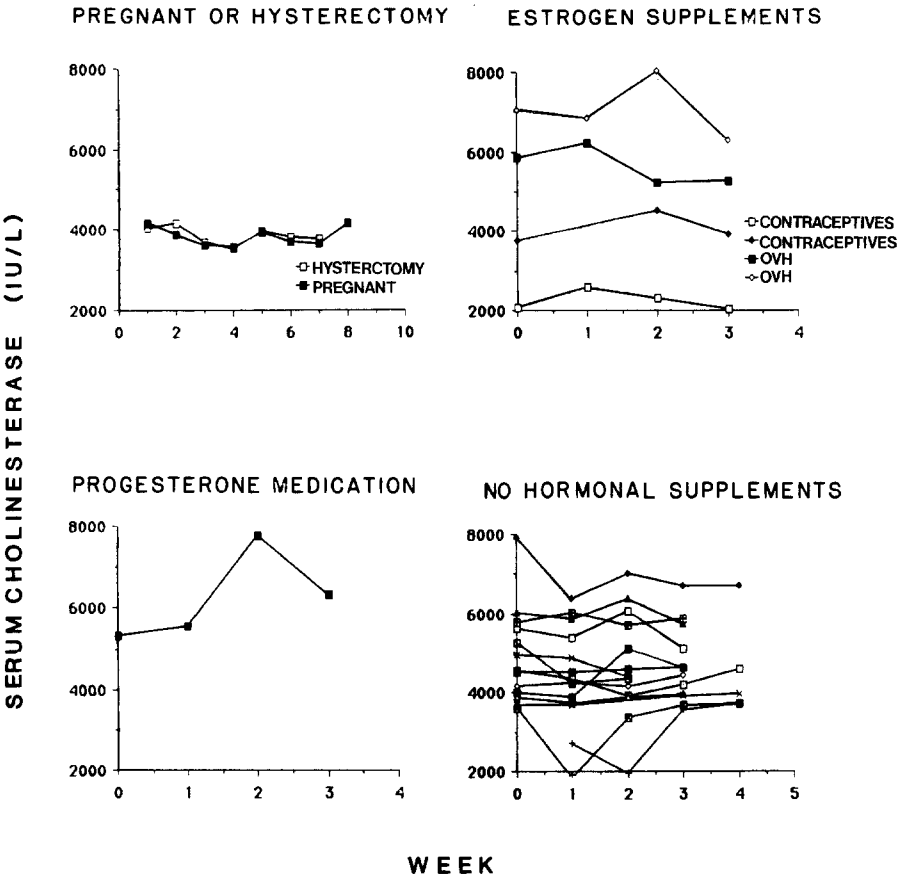

FIG. 2. Variation in serum cholinesterase activity (IU/liter) during the menstrual cycle for women on varying types of hormone therapy. Mensis occurs at week 0; each data point represents an average of two cycles.

Kaminskis, 1975). However, the results of this study do not support this hypothesis. Oral contraceptives and pregnancy were correlated with lowered serum ChE values but there was no correlation between progesterone and ChE or between week of the menstrual cycle and ChE. Women on birth control medication and women who had had an ovariohysterectomy and were taking oral estrogen medication both had progesterone values consistently below 1.0 ng/ml. Yet both groups of women had considerable variability in their ChE values (mean % CV = 12.0 and 8.8, respectively) and there was a twofold difference between their mean values (3160 and 6254 IU/liter, respectively). Additionally, intake of oral progesterone by one intact, normal woman for 13 days did not change her ChE values significantly.

Experiments by Illsley and LaMartinier (1981) suggest that ChE levels in female rats are influenced by estrogen. Ovariectomized rats had significantly lower ChE values than did intact females and replacement therapy with estrogen increased

TABLE 2
VARIABILITY OF SERUM, PLASMA, AND RED BLOOD CELL (RBC) CHOLINESTERASES

	Number of subjects	Cholinesterase		
		Serum	Plasma[a]	RBC[a]
A. Age Class				
25–30	7	31.5[b]	41.6	16.4
31–35	6	19.6	32.5	1.1
36–40	4	30.9	—	—
41–55	4	29.8	—	—
B. Age Class				
25–30	7	11.9 (2.3)[c]	6.9 (0.2)	7.5 (1.8)
31–35	6	7.4 (0.6)	6.6 (1.2)	6.6 (2.6)
36–40	4	17.4 (5.7)	—	—
41–55	4	6.8 (1.2)	10.5 (—)	7.1 (—)

Note. (A) Among individuals within each age class (expressed as the percent coefficient of variation of the mean of the individual means). (B) Within individuals in each age class (expressed as the mean of the percent coefficient of variation for all women within each group).

[a] Number of subjects = 2, 2, and 1 for age groups 25–30, 31–35, and 41–55, respectively.

[b] Number of subjects.

[c] Standard error of the mean. N = number of subjects.

ChE levels to 70% of normal. The two women in the present study that had been ovariectomized and were taking exogenous estrogen had the highest ChE values of the women in the study. This suggests that estrogen may be a powerful stimulator of ChE production in humans as well. However, this theory is rebutted by

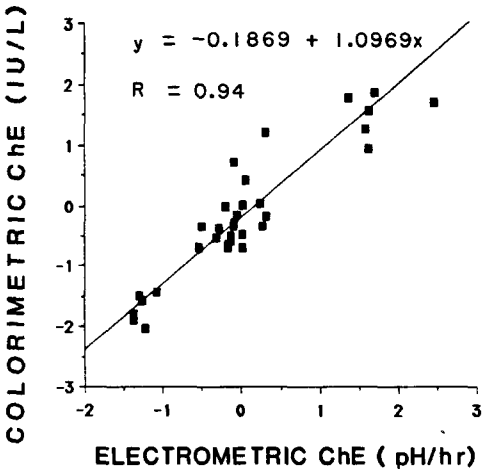

FIG. 3. Scatter plot showing the correlation between serum cholinesterase activity measured colorimetrically (IU/liter) and plasma cholinesterase activity measured electrometrically (pH change/hr) for five women in the study. Ages of the women ranged from 28 to 46 years; two of the women were taking oral contraceptives. Data standardized prior to analysis to the mean equal to zero and standard deviation equal to one.

the observations of ourselves and others (Robertson, 1967; Sidell and Kaminskis, 1975) that women taking oral contraceptives had ChE values significantly lower than the norm. It may be that estrogens contained in oral contraceptives function differently than endogenous estrogen in ChE regulation. The contraceptive used by the women in this study contained norethindrone and ethinyl estradiol.

Age is known to significantly affect ChE values with postmenarchial women having significantly lower ChE values than younger women (Sidell and Kaminskis, 1975). In the present study, ChE values of older women (age group 41 to 55 yrs) were not significantly different from values of 31 to 35 year olds (Table 1). However, this probably was due to the fact that two out of four of these women were ovariectomized and taking estrogen medication and the other two women were premenopausal.

It is interesting to note that measurements of serum ChE activity performed colorimetrically were correlated 1:1 with plasma ChE activity measured electrometrically (Fig. 3). This confirms that the two methods measure the same enzyme activity and can be used interchangeably, although the electrometric method appears to be slightly less variable. It is not surprising that RBC ChE activity is not correlated to serum or plasma ChE since RBCs contain acetylcholinesterase while human serum contains primarily butyrylcholinesterase (Willis, 1972). Consequently, the two assays are measuring different enzymes that may have different activities and degrees of responsiveness to hormonal control. The significant negative correlation between serum and RBC ChE exhibited by one of the two women on oral contraceptives (Fig. 1B) may be an artifact of the small sample size or may be due to the extremely low levels of serum ChE in women on this type of hormone therapy.

CONCLUSIONS

In summary, there is considerable variation in serum ChE values both within and among normal women. This variation probably is due to the influence of some sex steroid hormone since pregnancy, oral contraceptives, estrogen therapy, and ovariectomy all affect serum ChE values. However, the relationship between the enzyme and hormone concentration is not a simple one-to-one correlation. Rather, it is likely that there is some interaction of these hormones with each other (and perhaps others such as thyroxin [Illsley and LaMartinier, 1981]) in how they influence the production and/or degradation of ChE. This study design was not sophisticated enough to examine these types of interactions. The study did show that there is a large amount of variation of ChE values within some normal women (up to 32%) regardless of age or hormone therapy. Serum ChE values in other women are less variable (less than 9%). Therefore, interpretation of ChE tests used in pesticide monitoring operations and medical situations must be done with caution if based on a single measurement rather than as a comparison to several (at least four or five) previous tests conducted on the same subject. If testing is limited to once or twice a year, the best approach would be to compare values obtained to the mean of values reported for other women of similar age and hormone therapy groups. If the value falls within two standard deviations of the reference mean then the woman should be considered unexposed even though her

individual values may vary 50% from year to year. The amount of normal variation within an unexposed individual woman is too great to rely on comparisons between one or two yearly samples.

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