Carotene and Vitamin A in Pregnancy and the Early Puerperium.* (27376)

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Many reports exist describing carotene and vit. A levels in plasma during pregnancy and the puerperium(1-6). In general, it has been observed that carotene increases and vit. A decreases as pregnancy progresses toward term, while during the puerperium these components return to normal non-pregnant values.

Plasma levels of carotene and vit. A in the puerperium are usually reported as the average of several determinations made on different individuals, which are grouped by postpartum day. Treatment of data in this manner may lead to erroneous conclusions because of individual variation(7). The advantage of using daily serial determinations for each patient and then averaging values to describe the changes that occur has been neglected by most investigators. This approach has been used in the present study which was initiated to better describe the changes occurring in plasma levels of carotene and vit. A in the early puerperium.

Materials and methods. A total of 59 patients are reported in this study, and are divided into non-pregnant, pregnant and postpartum groups. Of this number, analyses were made on 20 non-pregnant, 25 pregnant, and 21 postpartum patients. Ten of the patients reported in the pregnant group were followed through the puerperium thus accounting for the apparent larger number of individuals studied. The patients were of comparable age and well nourished, and blood specimens were obtained and analyzed during the month of July.

Carotene and vit. A levels for the pregnant group were determined within 48 hours of delivery, while puerperal patients were analyzed daily through the fifth postpartum day after an uncomplicated labor and vaginal delivery. Blood samples were collected in heparinized tubes after an overnight fast, except for 15 patients in the pregnant group, which were taken just prior to delivery. Five milliliters of plasma were used for each determination.

Carotene and vit. A were determined according to the procedure of Kimble(8) using an Evelyn colorimeter. Vit. A acetate (General Biochemicals) containing one million units per gram and crystalline beta carotene (Nutritional Biochemicals Corp.) were employed as standards. Results are expressed in micrograms per 100 ml plasma, and significant differences between means were determined by the Fisher "t" test.

Results. Plasma levels for carotene and vit. A with their standard deviations are shown for the groups studied in Table I. The data show that average plasma values for carotene were higher and for vit. A lower in the pregnant than in the non-pregnant group, and both differences were significant at the .1% level. Following delivery and by the first postpartum day, the puerperal group showed a decrease in carotene and an increase in vit. A, significantly different ($P<.005$) from those found in the pregnant patients.

Though the values for the puerperal patients were lower than those observed in the non-pregnant group, all were not statistically different, but were variable. Hence when the means of these 2 groups were evaluated a difference was found in carotene for the third and fourth postpartum days, but none for the first, second and fifth; vit. A showed a difference on the first, fourth, and fifth postpartum days, but none for the second and third. Further, no significant differences were found between postpartum days for either carotene or vit. A, which appeared to remain unchanged throughout the puerperium.

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TABLE I. Means and Standard Deviations of Carotene and Vit. A Expressed as μg per 100 ml of Plasma, the Corresponding Coefficient of Variation, and Carotene to Vit. A Ratio for Non-pregnant, Pregnant and Puerperal Groups.

Group	No. of patients	Carotene μg/100 ml plasma	Carotene Coefficient of variation†	Vit. A μg/100 ml plasma	Vit. A Coefficient of variation	Carotene /Vit. A
Non-pregnant	23	148 ± 30*	21	38 ± 8	22	3.9
Pregnant	25	169 ± 56	33	22 ± 5	22	7.7
Postpartum day—1	21	128 ± 38	45	30 ± 9	30	4.3
2	20	122 ± 47	38	32 ±10	32	3.8
3	20	114 ± 25	22	33 ± 9	27	3.5
4	20	115 ± 42	36	29 ± 8	27	3.9
5	15	129 ± 42	33	30 ± 8	25	4.3

* Mean ± stand. dev. † Coefficient of variation = Stand. dev./mean × 100.

Carotene was more variable than vit. A in both the pregnant and puerperal patients in contrast to the values for the non-pregnant group. Table I shows the coefficients of variation (standard deviation/mean × 100) for all groups analyzed and it is seen that carotene is about twice as variable in pregnancy and the puerperium as vit. A. No difference was observed in the variation for vit. A between non-pregnant and pregnant patients, and only a slight increase was seen in the puerperium. In addition, the variation in vit. A in the puerperium appears to return to the magnitude of the non-pregnant group by the fifth postpartum day, while carotene remains essentially unchanged.

The ratios for carotene to vit. A (C/A) are calculated for the 3 groups (Table I). The mean ratios for non-pregnant (3.9) and puerperial patients (4.0) were comparable and differed significantly ($P<.005$) from those observed in the pregnant group (7.7).

To assess the effects of dietary variation the pregnant subjects were divided into staff ($N = 12$) and private ($N = 13$) patients. The carotene and vit. A levels found for these 2 groups were not considered to be different ($P>0.2$).

Discussion. These data confirm previous reports(1-6) that changes occur in carotene and vit. A content of plasma during pregnancy. These changes are reflected as increased carotene and decreased vit. A, and attain their maximum values at term(3). As shown here they are significantly different from those found in non-pregnant and postpartum patients. The values found for vit. A, however, appear to be in better agreement with those in the literature(1-3) than those observed for carotene. This would be expected from the coefficients of variation shown in Table I indicating that plasma vit. A is less variable than carotene during pregnancy and the puerperium.

Changes observed in carotene and vit. A at term are probably not due to diet as significant differences in plasma levels are not apparent when staff and private patients are compared. The fact that all samples were taken and analyzed in the month of July tends to reduce differences due to season(9). Bodansky(3) reported that plasma levels of carotene and vit. A are affected only by extremes in dietary intake of these components, and Lund(2) showed that these levels reflect dietary intake, but tend to remain unchanged within the same dietary group. Darby(9) is in agreement with these effects of diet, but indicated that the carotene level is influenced to a greater extent by differences in carotene intake than vit. A. It thus appears that though two diverse groups are analyzed, the results presented are from individuals within the same dietary group, and it is unlikely that diet was responsible for the changes observed in these experiments.

Postpartum values show an immediate drop in carotene and an increase in vit. A which occurs by the first postpartum day and remains essentially unchanged through the fifth postpartum day. This rapid change agrees with the work of Barnes(5) who reported carotene and vit. A values on a serial basis for 6 days in the puerperium, but the abso-

lute values recorded by Barnes are low and do not agree with those shown here or in the literature(1-6). The data also agree with that of Lund and Kimble who described(2) this immediate change in vit. A as occurring within 6-24 hours following delivery, but disagrees in that their data show a progressive increase for vit. A through the second puerperal day with little change in carotene. Lubke and Finkbiemer(6) followed puerperal changes through the fourth postpartum day and demonstrated a 2-fold increase in vit. A. From the results presented here, it is apparent there is no progressive increase or decrease in the components, and if one does occur, it is believed to be a fortuitous event.

These changes observed in the puerperium for carotene and vit. A are significant when compared to the pregnant group, but the significance is variable when compared to values for non-pregnant patients. Since a real difference between means for the puerperal, and non-pregnant groups cannot be obtained with any regularity, no correlation can be made relating carotene and vit. A levels to the postpartum day. This may account for the disagreement among investigators reporting puerperal values of plasma carotene and vit. A. Though the results are not compelling, the evidence supports the conclusion that changes in carotene and vit. A following delivery are rapid, significantly different from pregnant values, and differences observed between the puerperal and non-pregnant groups are probably due to chance because of the increased dispersion about the means (see coefficient of variation in Table I). Consequently, the C/A ratio calculated for the non-pregnant, pregnant and puerperal patients was of interest. The results in Table I show that an inverse relationship apparently exists and supports the conclusion of Darby (9), Bodansky(3), and Anderson(10) who showed such a relationship. The constancy of the ratio for the non-pregnant and puerperal patients appears to be a better indication of the immediate changes in carotene and vit. A following delivery and their return to a ratio characteristic of the metabolism found in non-pregnant patients. Further, the constancy of this ratio indicates that it may be a better parameter for detecting changes in carotene and vit. A metabolism than absolute values, and offers more evidence for the conclusion that differences observed between non-pregnant and puerperal groups are not real. Finally, it appears that differences in plasma carotene and vit. A which occur during pregnancy involve changes in the metabolism of both components as both show significant differences from those values found for the non-pregnant and puerperal groups.

The association of the changes observed in carotene and vit. A in pregnancy and the puerperium with increased lipids as suggested by Darby(9) is not without basis as the hyperlipidemia of pregnancy has long been recognized(11). Plasma lipids increase from the second trimester to term and like carotene decrease in the puerperium; however, a longer time is required for their return to non-pregnant(12) levels. More recently Burt (13) showed that the plasma free fatty acids (FFA) also increased during pregnancy, but unlike the other lipids, they return to pre-pregnant levels by the second postpartum day, a sequence very similar to the rapid changes observed here with carotene and vit. A. The work of Varandani(14) who demonstrated that complete extraction of vit. A required treatment with alkali suggests that the metabolism of vit. A and probably carotene is more closely related to the metabolism of FFA and lipids than previously suspected.

Summary. 1. Levels of carotene and vit. A in pregnant patients were shown to be significantly different from those found in non-pregnant and puerperal patients. 2. Differences in the means of carotene and vit. A between puerperal and non-pregnant patients were variable and it appeared that changes in carotene and vit. A following delivery are rapid and not significantly different from those found in the non-pregnant group. No differences were found in carotene and vit. A between postpartum days. 3. Diet was apparently not a factor influencing carotene and vit. A levels in this study. 4. Evidence was given supporting the inverse relationship between carotene and vit. A and it was suggested that the ratio (C/A) of these components may be a better parameter for de-

tecting changes occurring in their metabolism. 5. The possible association of vit. A and carotene changes with changes in lipids during pregnancy was considered.

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Adenovirus Plaque Formation in Grivet Monkey Kidney Cells. (27377)

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The plaque technic developed by Dulbecco (1) provides a method which is suitable for quantitative analysis of infectivity of virus preparations, isolation of pure lines of virus, and differentiation of virus strains and types (2,3). Plaque formation by adenovirus type 2 in stable cell lines was demonstrated by Bonifas and Schlesinger(4) and types 4 and 5 by Kjellén(5). This report describes the adaptation of the plaque technic to adenovirus types 1 through 7 in monolayers of grivet monkey kidney cell cultures (*Cercopithecus aethiops*).

Materials and methods. Tissue culture. Grivet monkey kidney cell cultures were prepared in Smith bottles by a method previously described(6).

Viruses. The strains of adenovirus types 1 through 7 used were of human origin. They were isolated in human embryonic cell cultures and subsequently adapted to monkey kidney cells and are strains employed routinely in this laboratory. All of the virus samples described here were obtained from adenovirus infected fluids of grivet monkey kidney cell cultures.

Agar overlay medium A was prepared as described previously(6), with 2 exceptions. The neutral red was omitted from the overlay and a solution of *protamine sulfate* was added to yield a final concentration of approximately 400 μg/ml.

Agar overlay medium B was prepared in the same manner as agar overlay medium A, except that neutral red was added to yield a final concentration of 1:25,000.

Virus seeding. The medium was removed from each of the cell culture bottles. The cell sheets were washed once with Hanks' balanced salt solution adjusted to pH 8.0. Bottles were seeded with appropriate dilutions of virus at 0.5 ml per bottle. Hanks' balanced salt solution containing 0.1% *bovine serum albumin* $\left(\frac{W}{V}\right)$ was used as the virus diluent. The bottles were placed in the 36°C incubator for 1.5 hours. At the end of the incubation period, the cell sheets were overlaid with 15 ml of the agar medium (A, above). After the agar had hardened, the bottles were incubated at 36°C in an inverted position. At the end