Studies of marginal zinc deprivation in rhesus monkeys. I. Influence on pregnant dams

Mari S Golub, M Eric Gershwin, Lucille S Hurley, Deborah L Baly, and Andrew G Hendrickx

ABSTRACT Rhesus monkeys were fed a diet marginally deficient in zinc (4 ppm zinc) throughout pregnancy and were monitored for changes in hematological, biochemical, and immunological parameters. This dietary zinc level was chosen because it did not produce an overt deficiency syndrome when fed for 10 wk to nonpregnant monkeys. Deprived animals were compared to control groups fed a zinc replete (100 ppm) diet ad libitum or on a food restricted (pair fed) basis. Beginning in the 3rd trimester zinc-deprived monkeys exhibited characteristic signs of deficiency including dermatitis, anorexia, and low levels of plasma zinc (less than 65 μg/100 ml) and of serum alkaline phosphatase activity. The extent of plasma zinc depression in deficient monkeys was dependent on total food intake; severely anorexic monkeys lost weight but maintained normal plasma zinc levels; monkeys that gained 20 to 30% of their body weight during pregnancy had severely depressed plasma zinc. Plasma vitamin A was reduced in the deprived group while copper, magnesium, and folate levels remained similar to controls. Hematological changes indicative of iron deficiency anemia (reduced packed cell volume, mean corpuscular volume, and Hb) were also seen in severely deficient monkeys. In addition, the peripheral lymphocyte mitogen response was reduced in deficient dams. We conclude that marginal deficiency of dietary zinc can produce significant abnormalities of nutritional status and has the potential for producing serious immunohematological dysfunction during pregnancy. Am J Clin Nutr 1984;39:265-280.

KEY WORDS Zinc deficiency, dietary zinc, rhesus monkey, pregnancy, immunology, hematology, anorexia, trace metals

Introduction

Pregnancy is a period of marked anabolism. It is not surprising therefore that zinc deprivation, which influences nucleic acid and protein biosynthesis, has been reported to alter the course of fetal development (1–3). For example, during early stages of gestation, i.e., organogenesis, deficient zinc intake is associated with a wide range of teratogenic anomalies in rats (2, 4–6). However, the metabolic demands of pregnancy are considerably less in large mammals, and primates in particular (7), than in rodents. In addition, the degree of zinc deficiency that produces the frequent and varied congenital anomalies in animal studies seldom occurs in human populations. Although a link between zinc deficiency and poor pregnancy outcome has been suggested by some human studies (8–11), the importance of zinc deficiency as a risk factor in human pregnancy has not been established.

To help resolve these issues we have begun a long-term study designed to define the

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consequences of zinc deprivation in pregnant rhesus monkeys and their offspring. Severely zinc-restricted diets have previously been found to produce clinical signs of zinc deficiency in squirrel monkeys, marmosets, and rhesus and bonnet macaques (12–15). The dietary zinc level used in this study does not produce overt clinical signs of zinc deficiency in nonpregnant monkeys but does produce a 20 to 30% decrease in plasma zinc, a level previously defined as "marginal deficiency" (16–18). We report herein that marginal zinc deficiency, even when begun after conception, has a marked influence on maternal health. These observations have significant implications for human populations.

Methods

Subjects

Female rhesus monkeys (Macaca mulatta) were obtained from a breeding colony of healthy, multiparous rhesus monkeys, ranging in age from 7 to 15 yr, maintained at the University of California Primate Research Center.

Housing of animals

The housing and environment of the animals was designed to minimize contamination with extraneous zinc (19, 20). Monkeys were housed individually in stainless steel cages, previously cleaned to remove zinc; each room had controlled lighting and temperature. Deionized distilled water was available ad libitum in plastic containers with neoprene stoppers; fluid intake was monitored daily. Food was given in stainless steel containers designed to minimize both spillage of the purified diet and zinc contamination. All experimental materials and animals were handled with individually wrapped, single-use polyethylene gloves.

Animal housing and maintenance procedures met guidelines established by the Federal Animal Welfare Act and the Institute of Laboratory Animal Resources. The California Primate Research Center is accredited by the American Association of Laboratory Animal Sciences.

Mating and establishment of pregnancy

The monkeys were bred with normal rhesus males, according to the methods of Hendrickx et al (21). Menstruation was checked daily by visual examination of vaginal swabs, and the midcycle was calculated individually for each monkey based on menstrual cycle history. The female was mated with a single male every other day for 3 days during midcycle. The mating was limited to 2 h/day, with day 13 of the cycle designated as day 0 of pregnancy. Pregnancy was confirmed at 17 to 23 days postconception by the monkey chorionic gonadotropin assay, which involves a hemagglutination inhibition test in the urine, or radioreceptor test in blood.

Diets and feeding

The composition of the diet was based on previous studies which showed that similar diets supported normal growth and reproduction in rhesus monkeys (15, 16). In the present studies, sprayed egg white was used as the protein source and the diet was enriched with additional biotin. The diet was prepared and pelleted commercially (Dyets, Inc, Bethlehem, PA). Its composition is shown in Table 1. The concentration of zinc was determined in all batches of the diet in our laboratory by atomic absorption spectrophotometry (see below). Zinc levels of 100 and 4 μg/g were chosen to represent control and marginally deficient zinc intake. We have shown previously that feeding a diet containing 4.0 μg/g zinc for 6 wk resulted in a 20 to 40% reduction of plasma zinc below the level observed in control rhesus monkeys (17). Because zinc deficiency has been shown to cause inanition, one group of monkeys was fed the control diet in amounts equal to the measured intake of the animals fed the deficient diet. In this way, it is possible to distinguish those alterations attributed to zinc deficiency per se from those due to the concomitant inanition and consequent caloric deprivation. Animals in the food restricted (pair fed) group were matched one-for-one for age and weight to animals in the zinc-restricted group and pair-fed to the matched animal. Daily rations for the animals in the food-restricted group were calculated using the standard metabolic weight conversion factor which consisted of the animal’s preexperimental weight in kg⁰.⁴ divided by the zinc deprived animal’s preexperimental weight in kg⁰.⁴.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Composition of diet for monkeys (g/kg)</th>
</tr>
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<tr>
<td></td>
<td></td>
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<tr>
<td>Spray dried egg white</td>
<td>200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>586</td>
</tr>
<tr>
<td>Corn oil</td>
<td>80</td>
</tr>
<tr>
<td>Salt mix*</td>
<td>40</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin mix†</td>
<td>20</td>
</tr>
<tr>
<td>Fiber</td>
<td>70</td>
</tr>
<tr>
<td>Banana flavoring‡</td>
<td>2</td>
</tr>
</tbody>
</table>

* Composition of basal salt mix, in g/kg of mix: CaCO₃, 300; Ca(H₂PO₄)₂·H₂O, 110; K₂HPO₄, 325; NaCl, 170; MgSO₄, 60; FeSO₄·7 H₂O, 32.5; MnSO₄·H₂O, 2.3; KI, 0.8; CuSO₄·5 H₂O, 0.3; Na₂SeO₃·5 H₂O, 0.00075. (Further supplements of 0.0325 g/kg NaH₂SeO₃ and 0.044 g/kg Cr₂O₃ were used during the second half of the study.)
† A mixture of crystalline vitamins in glucose providing the following amount per 100 μg diet: in mg: choline chloride 200; ascorbic acid, 100; inositol, 50; niacinamide, 25; calcium pantothenate, 10; menadione, 5; thiamin HCl, pyridoxine HCl, and riboflavin, each 3; p-amino benzoic acid, 1; folic acid, 0.5; biotin, 0.4; cyanocobalamin, 0.006; and retinyl palmitate, 1500 IU; cholecalciferol, 150 IU; and α-tocopheryl acetate, 30 IU.
‡ Paccoseal imitation banana flavoring.
ZINC DEFICIENCY IN PREGNANT MONKEYS

The scheduling of diets was as follows: 1) before the time of mating, all monkeys were fed the control diet (100 ppm zinc) ad libitum for 4 wk to allow for adjustment to the diet and dietary routine; 2) at the time of conception, all monkeys began consuming the diet for the group to which they had been assigned, ie, control ad libitum, control food restricted, or marginally deficient. The diets were fed during gestation and lactation (to 5.5 months postnatal).

In assigning monkeys to the three groups, they were matched for age, body weight, parity, and years in the colony. Each animal was fed a preweighed (75 g) food portion, individually bagged, twice daily at 0930 and 1400. Any animal consuming its full ration for 3 consecutive days was then given an additional 75-g portion at the morning feeding. Any monkey that ate no food for 4 successive days was reported to the veterinary staff for evaluation of anorexia, and was then followed for signs of dehydration or depression that might indicate the necessity of therapeutic intervention. One pair-fed monkey was removed from the study in the 1st trimester because of severe anorexia and weight loss; two other animals received brief oral electrolyte supplementation.

Experimental procedures

The monkeys used in the study were as follows: 1) eight female rhesus monkeys, impregnated, fed the control diet ad libitum during gestation and lactation; 2) 11 female rhesus monkeys, impregnated, fed the control diet in restricted amounts during gestation and lactation (ie, pair-fed to the animals in group 3); 3) 15 female rhesus monkeys, impregnated, fed a diet marginally deficient in zinc during gestation and lactation.

The present report summarizes the effects of these treatments during pregnancy; subsequent papers will present information on the offspring and the postnatal period.

At 0, 45, 90, 135, and 150 days of gestation, and at delivery, blood samples were obtained from 6 to 15 monkeys in each experimental group for: 1) complete blood counts; 2) serum sodium, potassium, chloride, CO₂, blood urea nitrogen, creatinine, calcium, phosphorous, SGPT, SGOT, lactic dehydrogenase (LDH), γ-glutamyl transferase, alkaline phosphatase, uric acid, protein, albumin, cholesterol, and triglycerides (22); 3) plasma zinc, copper, iron, and magnesium (see below) 4) red cell and serum folate (day 135 only) (23); 5) plasma vitamin A and retinol binding protein (day 135 only) (see below) 6) serum immunoglobulins IgG, IgM, and IgA (see below); 7) hemolytic complement (see below); 8) percentage of E rosette and surface Ig* cells (day 135 only) (see below); 9) peripheral blood lymphocyte responsiveness to phytohemagglutinin (PHA-P), Concanavalin A (Con A), and pokeweed mitogen (see below). Additionally, at 30 and 100 days of pregnancy, hair samples were obtained for trace metal analysis. Finally, on day 100, amniocentesis was performed for amniotic fluid samples.

Fasting blood samples were obtained between 10:00 and 11:30 AM (post-delivery term) samples were obtained at approximately 1:00 PM from the cephalic vein of unanesthetized monkeys. Samples for analysis of trace metals were drawn using trace element-free needles and syringes and zinc free heparin. Amniotic fluid samples were obtained from dams anesthetized with ketamine (Vetalar; Parke-Davis, Morris Plains, NJ). Hair was obtained by clipping a section of about 5 cm² from the subcapsular area; the hair was wrapped in plastic for storage before analysis.

Diet intake and health surveillance

Food and water intake were recorded daily throughout pregnancy and summarized during consecutive 30-day periods. Stool consistency and general health were monitored each morning. Because of the known influence of zinc deficiency on epidermal tissues, specific examinations to monitor alopecia and dermatitis were carried out before pregnancy, on day 30 and 100 of pregnancy, and 1 day after delivery. Alopecia was evaluated by an unbiased observer. The location and size of areas lacking hair was also recorded. Dermatitis was recorded in two categories, rash or red raised papules, and dry, flaky dermatitis. Animals were weighed before pregnancy and at 30-day intervals throughout pregnancy. In addition skinfold thicknesses at four identical sites (triceps, thigh, subscapular, and suprailiac) were measured using a Harpenden skin caliper (Quinton Instruments, Seattle, WA) at 30 and 100 days of gestation and at 1 day postdelivery (24).

Trace element analysis

All glassware was washed with mild detergent and rinsed with distilled deionized water. The glassware was then placed in 20% nitric acid for 48 h and rinsed with several volumes of distilled double deionized water. Rinsed glassware was placed in a covered polyethylene drying basket and stored in sealed plastic bags before use. Trace element analysis was performed on frozen plasma and on amniotic fluid within 48 h of sample collection. Hair samples were subjected to a washing-extracting procedure before acid-washing and analysis. Hair was wrapped in Whatman no 40 filter paper, tied with a thread, and washed in 0.1% (w/w) Ivory liquid detergent in an Erlenmeyer flask with occasional agitation over several hours and then rinsed with deionized water until no foaming was noted. The samples were then washed twice with 95% ETOH, once with ethyl ether, and allowed to air dry in an oven at 100°C. Samples were stored in a desiccator until analyzed.

The processed plasma, amniotic fluid, and hair samples were weighed and wet ashed with 16 N nitric acid, concentrated by evaporation, and diluted with 0.1% lanthanum oxide in 1 N nitric acid. Elemental analysis was performed on a Perkin-Elmer 370 atomic absorption spectrophotometer (Norwalk, CT) using a 3-s integration time, a Boling burner, and an air-acetylene flame. Concentrations were calculated from the absorbance values by use of linear regression equations (25).

Vitamin A analysis

Vitamin A was determined in blood samples on days 0 and 135 of gestation. Care was taken to avoid exposure of the blood to light during venipuncture and thereafter. After centrifugation, plasma was kept frozen at −20°C for up to 2 months before analysis. To confirm stability,
plasma vitamin A levels were checked during this 2-month period; values on four representative monkeys were 19.50, 33.24, 29.29, and 30.86 μg at time 0 and 21.35, 32.52, 28.42, and 35.95, respectively, when analyzed 2 months later. The variation observed is within the accuracy of the assay. Vitamin A was measured using the fluorometric method of Kahan (26).

**Immune response**

The percentage of peripheral blood E rosette positive and surface Ig* cells was quantified as described previously (27). Blood lymphocyte responsiveness to mitogens was determined as follows: after isolation from peripheral blood samples, lymphocytes (2 x 10⁶) were distributed in triplicate in sterile U-shaped microtiter plates with either media, PHA-P, 1 μg/ml Con A, 0.2% pokeweed mitogen, or pokeweed mitogen, 0.2% v/v. These mitogen concentrations have been previously demonstrated to produce optimal stimulation. The plates were then incubated at 37°C in a 5% CO₂, 95% humidified atmosphere for 72 h. Four hours before harvest, 1 μCi of [³H]-thymidine was added and the cultured cells collected, using an automated multiple sample harvester (28). Serum IgM, IgG, and IgA concentrations were measured before initiation of the study and at the end of the study period by radial immunodiffusion using heavy chain specific antisera and reference standards (29). Hemolytic complement was quantified as previously described (29).

**Statistical analysis**

All parameters were initially evaluated with a 3 x 5 repeated measures analysis of variance (regression solution) (30). The factors in the analysis were: groups (zinc deprived, ad libitum control, and food restricted control); and gestation day (0, 45, 90, 135, and delivery). Additional tests of differences between groups were based on a one-way, three group analysis of all data available at a given time point. Analysis of variance was used for most parameters. Nonparametric tests were used for data from rating scales and for some postdelivery data that were not normally distributed. For purposes of comparison, a normal range of values was determined for many parameters by constructing a 95% confidence interval derived from a sample of 27 to 41 nonpregnant rhesus females from the breeding colony.

**Results**

**Zinc deficiency syndrome**

No overt signs of zinc deficiency were recorded in the zinc-deprived monkeys during the first half of pregnancy. However, by midpregnancy components of the deficiency syndrome, such as reduced plasma zinc levels, anorexia, and dermatitis, began to appear. By late pregnancy and postdelivery, a deficiency syndrome was apparent in most of the deprived animals. Many of the zinc-deprived monkeys were anorexic and failed to increase their food intake and to gain weight during the 3rd trimester (Fig 1). Significantly fewer zinc-deprived animals (3/15) than ad libitum controls (5/8) showed at least 10% weight gain from prepregnancy to post delivery time points (Fishers exact probability test, p = 0.047).

By the time of delivery, the plasma zinc concentration in the deprived animals was less than 50% of the normal prepregnancy level and was significantly lower than that of the controls (Fig 2). Eleven of 15 deficient animals had dermatitis characteristic of zinc deficiency at delivery, as compared to 0/8 ad libitum controls ($x^2 = 5.88, p < 0.025$). Three of 10 pair-fed controls ($x^2 = 6.66$, p

![FIG 1. Decreased weight and subcutaneous fat in anorexic zinc-deficient monkeys and corresponding pair-fed controls. Measurements were taken prior to pregnancy and the day after delivery. Anorexic animals ate 2.58 ± 0.22 kg food per kg³¹4 body weight during pregnancy as compared to 4.53 ± 0.79 kg/kg³¹4 in normally eating animals. AL, ad libitum controls, clear bar; PF, pair-fed controls, striped bar; ZD, zinc-deficient group, black bar]. NS per group: AL = 8; PF, not anorexic = 6; PF, anorexic = 4; ZD, not anorexic = 11; ZD, anorexic = 4.
< 0.01) also were rated as exhibiting "noticeable" dermatitis; however, the lesions were not characteristic of zinc-induced dermatitis (major occurrence over joint areas, progression from raised papules to flaky encrusted areas) as compared to zinc-deficient group. No differences among the three diet groups were seen in frequency of loose stools or alopecia, as previously reported in zinc deficiency (31). Alopecia is common in pregnant rhesus monkeys and was seen in more than 50% of animals in all diet groups.

**Food intake and weight gain**

Food intake during pregnancy was not significantly lowered in zinc-deficient animals as a group. However, four of the 15 zinc-deprived animals showed severe anorexia, with low food intake, weight loss, and reduction in fat stores as indicated by skinfold measurements. Animals pair-fed to these anorexic monkeys also lost weight and subcutaneous fat (Fig 1). As discussed below, the degree of anorexia was an important factor in the clinical response to zinc deficiency. In particular, average plasma zinc levels during pregnancy were less severely depressed in anorexic animals (80 ± 8 μg/100 ml) than in nonanorexic (64 ± 5 μg/100 ml), presumably due to release of zinc into the circulation during weight loss and tissue catabolism (Fig 3). Anorexia in zinc deficiency was not attributable to age, initial body weight, or parity; these parameters were similar in anorexic and nonanorexic monkeys.

**Nutritional status**

Plasma zinc levels began to decrease in all animals at midgestation, but were markedly lower in zinc-deficient monkeys than in controls at the 3rd trimester sampling point (F = 9.48, p < 0.006) and after delivery (F = 14.74, p < 0.0001) (Fig 2). In addition, amniotic fluid zinc was lower in zinc-deficient animals than in controls at day 100 of gestation (F = 8.60, p < 0.02). Plasma copper concentration rose above the prepregnancy level in early pregnancy in all groups and continued to increase throughout pregnancy as previously noted in humans (32) (Fig 4). In contrast, magnesium concentration declined during pregnancy in all groups, also as noted in humans (33). Plasma iron concentration was somewhat elevated during pregnancy but did not move out of the normal prepregnancy range of values. No differences between diet groups in plasma copper, magnesium, or iron concentrations were observed over the experimental period.
No difference in trace metal content of hair samples was found.

Plasma folate, serum calcium, and phosphorous, assessed during the 3rd trimester, were not significantly different from pre-pregnancy values and did not differ in the diet groups (Table 2). A reduction in plasma vitamin A levels was observed in zinc-restricted monkeys late in pregnancy (135 days of gestation) (Table 2).

Serum levels of glucose, cholesterol, and triglycerides all showed distinctive time-dependent changes during pregnancy in normal animals (Fig 5). Glucose concentration declined gradually with a sharp drop in the postdelivery sample. Cholesterol decreased rapidly during early pregnancy while triglyceride levels rose sharply beginning in mid-pregnancy. Similar changes in cholesterol and triglyceride levels have been reported previously in pregnant rhesus monkeys (34–36).

Both zinc-deficient and food-restricted groups had glucose levels generally lower than controls during pregnancy. These differences were related to reduced food intake. Differences between the groups in glucose concentration were indicated by a significant interaction between diet and pregnancy in the repeated measures analysis of variance (F = 2.47, df 1, 27, p = 0.039). When the mean glucose concentration of pair-fed controls and zinc-deficient animals together was considered as one group, it was statistically lower than that of ad libitum-fed controls after delivery (F = 6.01, df 1, 27, p < 0.025). Sixty percent of the zinc-deficient and pair-fed animals had serum glucose levels under 35 mg/dl, while none of the ad libitum controls had glucose levels below the 95% confidence interval for nonpregnant animals (50 to 76 mg/dl).

A similar trend was seen for postdelivery serum cholesterol values; although statistically significant differences between groups were not found, 40% of animals in zinc-deprived and food-restricted groups had cholesterol levels below the 95% confidence interval for nonpregnant normal monkeys. Group differences in triglyceride level were also attributable to reduced food intake. Zinc-deprived and food-restricted animals had significantly lower triglyceride levels in the 3rd trimester (135 days of gestation) than ad libitum controls (F = 4.49, df 1, 26, p < 0.05). Triglyceride levels are usually elevated during late gestation in rhesus monkeys (36). Serum triglyceride concentration was lowest in monkeys that failed to gain weight during the 3rd trimester.

**Electrolytes**

Serum osmolality declined in early pregnancy in all groups by about 30% and remained low through parturition. This pattern has also been demonstrated in rats and
FIG 4. Changes in plasma levels of copper, magnesium, and iron during pregnancy. See legend Figure 2. No significant differences between groups were found at any time point.

in humans during pregnancy (37-38). Sodium values declined gradually during pregnancy, while potassium, chloride, bicarbonate, and anion gap were little changed. In general, electrolyte balance was maintained by all pregnant monkeys. One exception was a zinc-deficient monkey that had elevated levels of sodium, potassium, and chloride at the 135 day sampling point. Anion gap, osmolality, and packed cell volume were also elevated. The cause of the apparent dehydration and acidosis in this animal was
TABLE 2
Comparison of nutrient levels in three groups of rhesus monkeys before pregnancy and in the 3rd trimester (day 135 gestation)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Prepregnancy</th>
<th>Day 135 gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls, ad libitum</td>
<td>1.90 ± 0.03 (8)*</td>
</tr>
<tr>
<td>Serum calcium (ionized) (mEq/l)</td>
<td>Controls, food restricted</td>
<td>1.91 ± 0.03 (11)</td>
</tr>
<tr>
<td></td>
<td>Marginally zinc deficient</td>
<td>1.91 ± 0.03 (15)</td>
</tr>
<tr>
<td>Serum phosphorous (mg/100 ml)</td>
<td>Controls, ad libitum</td>
<td>4.10 ± 0.28 (8)</td>
</tr>
<tr>
<td></td>
<td>Controls, food restricted</td>
<td>4.48 ± 0.44 (11)</td>
</tr>
<tr>
<td></td>
<td>Marginally zinc deficient</td>
<td>4.65 ± 0.23 (15)</td>
</tr>
<tr>
<td>Plasma Folate (Lactobacillus casei activity:ng/ml)</td>
<td>Controls, ad libitum</td>
<td>12.9 ± 3.4 (3)</td>
</tr>
<tr>
<td></td>
<td>Controls, food restricted</td>
<td>10.6 ± 3.0 (4)</td>
</tr>
<tr>
<td></td>
<td>Marginally zinc deficient</td>
<td>16.8 ± 3.0 (9)</td>
</tr>
<tr>
<td>Erythrocyte folate (L casei activity:ng/ml)</td>
<td>Controls, ad libitum</td>
<td>50 ± 22 (3)</td>
</tr>
<tr>
<td></td>
<td>Controls, food restricted</td>
<td>50 ± 7 (8)</td>
</tr>
<tr>
<td></td>
<td>Marginally zinc deficient</td>
<td>50 ± 7 (8)</td>
</tr>
<tr>
<td>Plasma vitamin A (µg/100 ml)</td>
<td>Controls, ad libitum</td>
<td>55 ± 5 (8)</td>
</tr>
<tr>
<td></td>
<td>Controls, food restricted</td>
<td>49 ± 4 (10)</td>
</tr>
<tr>
<td></td>
<td>Marginally zinc deficient</td>
<td>47 ± 4 (14)</td>
</tr>
</tbody>
</table>

* Mean ± SEM (n).
† Significantly different from ad libitum group, t = 2.49, p < 0.05.

not determined but it did not persist at the next sampling point. Serum values for this animal at 135 days of gestation were excluded from the data analysis because of hemoconcentration.

Renal function

Serum levels of uric acid and urea nitrogen, as well as the blood urea nitrogen/creatinine ratio, did not change appreciably during pregnancy but were elevated by a factor of 1.5 to 2 in the postdelivery sample. During early pregnancy (45 and 90 days), serum uric acid levels were generally higher in the zinc-deficient and food-restricted monkeys (3.8 and 4.6 mg/dl) than in the ad libitum controls (2.4 mg/dl). This was reflected in a significant effect of diet (F = 3.81, df 2.27, p < 0.048) in the analysis of variance and was apparently related to decreased food intake. No effect of diet was found for urea nitrogen and blood urea nitrogen/creatinine.

Creatinine and bilirubin both declined significantly from prepregnancy to postdelivery sampling points (creatinine: 1.12 to 0.88 mg/dl; bilirubin: 0.26 to 0.20 mg/dl). No influence of diet group on these parameters was noted.

Serum enzyme activity

Changes in enzyme activity occurred in all groups at midpregnancy; alkaline phosphatase, LDH, and SGOT activities were lowest at this time while γ-glutamyl transferase and SGPT were elevated (Fig 6). However, in controls, enzyme levels generally stayed within limits of the normal nonpregnant range. In contrast, activity of the zinc metalloenzyme alkaline phosphatase was significantly lower in zinc deficient monkeys than in other groups at 135 days gestation (F = 16.42, df 1, 27, p < 0.01) and dropped below the normal range (Table 3). SGPT levels were also lower in zinc-deficient than in control animals in the third trimester (F = 6.22, df 1, 27, p < 0.025); SGPT is a pyridoxine-dependent enzyme, and has not previously been reported to be reduced in zinc-deficient states.

Levels of LDH, SGOT and SGPT were generally higher in pair-fed than in ad libitum-fed monkeys during the 3rd trimester and after delivery (Fig 6). Group differences were statistically significant for LDH (F = 6.75, df 1, 27 p < 0.025) and SGOT (F = 4.88, df 1, 27, p < 0.05) after delivery. This pattern suggests some degree of tissue dam-
with a marked depression in the postdelivery sample. The minor decline in these parameters can be attributed to increased plasma volume during pregnancy while the postpartum drop may be due to parturition associated blood loss.

Zinc-deprived monkeys with the lowest plasma zinc concentrations during pregnancy had hematological values indicative of anemia in the 3rd trimester (ie, packed cell volume values less than 40%, Hb less than 12 g/100 ml). Five of the 15 zinc-deprived animals fell into this category; plasma zinc concentrations in these monkeys were less than 65 μg/100 ml at both 90 and 135 days gestation (Table 4). After delivery, hematocrits were depressed and highly variable due to recent blood loss; however, mean corpuscular volume of the zinc-deficient group as a whole was lower than that of the controls (F = 8.76, df 1, 27, p < 0.01). This is suggestive of microcytic anemia. During the 3rd trimester or after delivery, abnormal red blood cell morphology (poikilocytosis, anisocytosis, leptocytosis) was seen more frequently in zinc-deprived animals (46%) than in food restricted or ad libitum controls (18 and 16%; χ² = 6.66 and 5.88, p < 0.025). In addition, Howell-Jolly bodies were common, as illustrated in Figure 7.

During pregnancy, the white blood cell count (WBC) was elevated above the prepregnancy range in both ad libitum-fed (AL) and zinc-deficient (ZD) monkeys (prepregnancy: AL = 7.2 ± 0.7 x 10³ WBC; ZD = 9.3 ± 2.9 x 10³ WBC; 90 days; AL = 13.3 ± 1.3 x 10³ WBC; ZD = 12.2 ± 2.2 x 10³ WBC). This trend was not seen in food-restricted monkeys, although the statistical interaction between diet and pregnancy was not significant (p = 0.095). Leukocytosis in pregnancy has previously been reported in primate species including humans (39, 40). The WBC dropped to normal range after delivery in the majority of the animals. However 53% of the zinc deprived monkeys maintained WBC values above the nonpregnant control range as compared to 26% of control animals (χ² = 7.82, p < 0.01). Analysis of the differential count showed a gradual increase in the ratio of neutrophils to lymphocytes during pregnancy, as seen in hu-

FIG 5. Changes in serum glucose, triglycerides and cholesterol during pregnancy. See legend Figure 2. Significant group differences are described in text.

Hematology

Red blood cell count, Hb, and hematocrit (PCV) all declined significantly but not dramatically during pregnancy in all groups,
The total serum protein level declined during pregnancy but remained in the same range for all diet groups (Table 5). The albumin/globulin ratio, which also decreased during pregnancy, was significantly lower in the zinc deficient than in the control groups at 135 days of gestation ($F = 7.25$, df 1, 27, $p < 0.025$). The low values were due in part to both elevated globulin and depressed albumin; five of 15 zinc-deficient monkeys had very low ratios (0.5 or less) during the 3rd trimester and postdelivery.
TABLE 4
Hematological parameters at 135 days gestation in zinc-deficient and control monkeys

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Red blood cell count (10^9/\mu l)</th>
<th>Hb</th>
<th>Packed cell volume %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, ad libitum</td>
<td>7</td>
<td>5.3 ± 0.2*</td>
<td>13.1 ± 0.5</td>
<td>40.8 ± 1.5</td>
</tr>
<tr>
<td>Controls, food restricted</td>
<td>11</td>
<td>5.6 ± 0.1</td>
<td>13.3 ± 0.4</td>
<td>40.9 ± 0.8</td>
</tr>
<tr>
<td>Mildly zinc deficient†</td>
<td>10</td>
<td>5.6 ± 0.1</td>
<td>13.3 ± 0.2</td>
<td>40.9 ± 0.8</td>
</tr>
<tr>
<td>Severely zinc deficient‡</td>
<td>5</td>
<td>5.1 ± 0.1</td>
<td>11.6 ± 0.48</td>
<td>37.1 ± 0.7¹</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
† Plasma zinc concentration 65 µg/100 g or higher in 2nd and 3rd trimesters.
‡ Plasma zinc concentration below 65 µg/100 g in 2nd and 3rd trimesters.
§ Significantly different from combined controls, \(t = 2.65, p < 0.01\).
|| Significantly different from combined controls, \(t = 2.50, p < 0.05\).

**Immune response**

Peripheral lymphocyte response to mitogens varied widely among individuals but was generally lower in zinc-deficient animals than in controls throughout pregnancy (Fig 8). Group comparisons (zinc-deprived versus pair-fed and ad libitum controls) were significant for all three mitogens at the 1st trimester sampling point (Con A F = 4.72, PHA F = 4.57, pokeweed mitogen F = 4.58; df = 1, 23 p < 0.05) and for Con A in the 3rd trimester (F = 5.41, df 1, 33, p < 0.05). (Mitogen response assays were not conducted after delivery due to the general instability of immunohematological parameters at this time and the desire to limit blood sampling.)

The immune response of deficient animals was also reduced relative to their individual prepregnancy base-lines. Thus 77% of Con A assays and 79% of PHA assays conducted in pregnant zinc-deficient animals were lower than base-line values as compared to 21 and 39% in controls (Table 6). Pokeweed mitogen response was least affected by zinc deficiency.

Immunoglobulin (IgG, IgA, and IgM) and complement levels did not show consistent pregnancy related changes and no influence of diet on these parameters was found. There were no significant differences in the percentage of E rosette positive cells when zinc-deprived animals were compared to either pair-fed or ad libitum control monkeys. Values in all groups ranged from 50 to 60%.

**Discussion**

Zinc deficiency in humans was first reported in populations of extreme poverty and very poor diets (43). In the last two decades, based on a variety of findings, concern regarding marginal zinc deficiency in developed societies has mounted substantially. Studies of several populations in the United States have suggested that prevalence of zinc deficiency may be greater than had been previously anticipated (44). Increased consumption of highly processed and refined foods may result in diets that are only marginally sufficient in zinc or even deficient (45–52). Pregnant and lactating women and rapidly growing infants and children are especially susceptible to zinc deficiency.

Recently, marginal zinc deficiency has been experimentally induced in humans (53–56) and this specific nutritional deficit was reflected in a variety of clinical and biochemical parameters previously shown to be sensitive to zinc dietary deprivation in animals. However, ethical and practical considerations prevent induction of experimental zinc deficiency in pregnant women.

The present data from zinc-deficient pregnant monkeys generally support the results of correlational studies in pregnant women (10, 47, 57, 58). In this regard, there were three major findings in the present study. First, dietary levels of zinc that do not produce signs of deficiency in nonpregnant monkeys or during early pregnancy can lead to a severe deficiency syndrome by late pregnancy. Second, the metabolic impact of zinc deficiency depends in part on whether or not anorexia is induced. Zinc-deficient animals that are anorexic lose weight and have lowered serum levels of glucose and triglycerides but maintain plasma zinc levels only slightly
FIG 7. Comparison of peripheral blood smears of a zinc deprived monkey (A, B) before pregnancy (before deficiency) and (C, D) after parturition. A high incidence of abnormal red blood cell morphology (poikilocytic cells) and Howell-Jolly bodies was seen in smears of zinc-deprived animals beginning in the 3rd trimester.

lower than those of controls. Deficient animals that continue to eat gain weight but have severely depressed plasma zinc levels. Third, a distinctive pattern of anemia (reduced Hb, hematocrit, and poikilocytosis) occurs during the 3rd trimester in monkeys with severely depressed plasma zinc levels.

The appearance of zinc deficiency syndrome in late pregnancy was accompanied by a drop in plasma zinc below 65 µg/100 ml, a level that is generally considered to represent a clinically relevant deficiency in humans (59, 60). Plasma zinc levels of con-
### TABLE 5
Serum protein levels in rhesus monkeys before pregnancy and in the 3rd trimester (day 135 gestation)

<table>
<thead>
<tr>
<th></th>
<th>Prepregnancy</th>
<th>Day 135 gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein (mg/100 ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls, ad libitum</td>
<td>7.6 ± 0.2 (8)*</td>
<td>7.7 ± 0.1 (8)</td>
</tr>
<tr>
<td>Controls, food restricted</td>
<td>8.0 ± 0.2 (11)</td>
<td>7.7 ± 0.2 (11)</td>
</tr>
<tr>
<td>Marginally zinc deficient</td>
<td>8.0 ± 0.2 (15)</td>
<td>7.9 ± 0.1 (15)</td>
</tr>
<tr>
<td><strong>Albumin (mg/100 ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls, ad libitum</td>
<td>4.40 ± 0.07 (8)</td>
<td>3.27 ± 0.25 (8)</td>
</tr>
<tr>
<td>Controls, food restricted</td>
<td>4.41 ± 0.12 (11)</td>
<td>3.33 ± 0.26 (11)</td>
</tr>
<tr>
<td>Marginally zinc deficient</td>
<td>4.44 ± 0.10 (15)</td>
<td>3.11 ± 0.30 (15)</td>
</tr>
<tr>
<td><strong>Globulin (mg/100 ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls, ad libitum</td>
<td>3.5 ± 0.11 (8)</td>
<td>4.4 ± 0.14 (8)</td>
</tr>
<tr>
<td>Controls, food restricted</td>
<td>3.9 ± 0.18 (11)</td>
<td>4.4 ± 0.06 (11)</td>
</tr>
<tr>
<td>Marginally zinc deficient</td>
<td>3.8 ± 0.10 (15)</td>
<td>4.8 ± 0.15 (15)</td>
</tr>
<tr>
<td><strong>Albumin/globulin ratio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls, ad libitum</td>
<td>1.16 ± 0.05 (8)</td>
<td>0.79 ± 0.03 (8)</td>
</tr>
<tr>
<td>Controls, food restricted</td>
<td>1.06 ± 0.07 (11)</td>
<td>0.79 ± 0.03 (11)</td>
</tr>
<tr>
<td>Marginally zinc deficient</td>
<td>1.11 ± 0.04 (15)</td>
<td>0.66 ± 0.03 (15)</td>
</tr>
</tbody>
</table>

* Mean ± SEM (n).
† Significantly different from combined controls, F = 7.25, p < 0.025.

---

**FIG 8.** Changes in peripheral lymphocyte response to mitogens during pregnancy. Mean ± SEM are shown. *Shaded area* represents 95% confidence interval derived from group of 42 nonpregnant female monkeys. Ad libitum and pair-fed controls are combined as no differences were found between these groups. All animals sampled at a given time point are included. Significant group differences are described in the text.

### TABLE 6
Changes in mitogen response during pregnancy relative to base-line response measures obtained before pregnancy; numbers of assays above and below individual base-lines are shown

<table>
<thead>
<tr>
<th></th>
<th>Con A</th>
<th>PHA</th>
<th>Pokeweed mitogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Above base-line</td>
<td>Below base-line</td>
<td>Above base-line</td>
</tr>
<tr>
<td>Zinc deficient</td>
<td>5</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Combined controls</td>
<td>22</td>
<td>6</td>
<td>17</td>
</tr>
</tbody>
</table>

χ² probability p < 0.002 p < 0.003 NS
trol animals demonstrate that in primates, as in humans (10, 59), zinc concentrations can fall below the normal range in late pregnancy despite adequate zinc nutrition. However, the results suggest that decreases below about 65 μg/100 ml cannot be considered "physiological" in late pregnancy in the rhesus monkey but rather probably represent a true deficiency state.

This study, as well as others including severe zinc deficiency (13, 15, 16), suggests that anorexia is not a consistent characteristic of zinc deficiency in primates. In contrast, anorexia is consistently seen in pregnant or growing rats and mice fed zinc-deficient diets (5, 18) as well as in the pregnant ewe (61). Nonetheless, the occurrence of severe anorexia in 25% of the zinc-deprived monkeys in the present study is noteworthy and suggests that metabolic factors that vary in genetically heterogeneous populations may predispose to zinc-induced anorexia. The fact that anorexic, zinc-deprived monkeys maintained "normal" plasma zinc levels for an extended period of time suggests that inadequate zinc nutrition can be masked by concurrent anorexia when plasma zinc is the sole index of zinc status. Recent studies in pregnant rats show that the catabolic state of the animal is important in the effects of dietary zinc deficiency. Restriction of food intake in rats fed a zinc-deficient diet ameliorated the deleterious effects of the zinc deficiency by increasing catabolism of maternal tissues and releasing zinc which was then available to the fetuses (62). It should be emphasized that in the mammal, the zinc concentration of maternal plasma is of major importance as a source for the embryo and fetus. To date, there are no studies of anorexia, food intake, and zinc status in pregnant women.

Abnormal hematological findings in severely zinc-deficient animals are particularly relevant to the human clinical situation. On the basis of clinical investigations, Jameson (10) proposed that some refractory anemias of pregnancy are due to zinc deficiency. Low serum zinc concentrations were found in the majority of 33 pregnant women whose anemia did not respond to iron, vitamin B₁₂, or folate. In addition, 13 of 20 pregnant women selected for very low serum zinc levels had Hb levels indicative of anemia (<11.0 mg/dl). Hematological data from both groups was consistent with increased intramedullary hemolysis and subsequent iron deficiency. Our findings of low Hb, and higher than normal incidence of abnormal erythrocyte shapes are also consistent with this hypothesis. This pattern of anemia could be related to the role of zinc in stabilization of cell membranes (63–65) or to its role in Hb synthesis (66). Recently, low Hb has also been reported in pregnant American women consuming marginally zinc-deficient diets (43).

Reduced immune responses of deficient pregnant monkeys is in accord with previous reports from this and other laboratories of immunodeficiency in zinc-deficient states (67). While mitogen response was lowered it is notable that less specific indicators of immune function such as lymphocyte count, immunoglobulin level, and complement were not affected. Cellular immunity and T cell function seem particularly sensitive to insufficient dietary zinc. In humans, correlations between zinc deficiency and reduced immune function have been reported in several clinical populations (68–71) but have not as yet been assessed in pregnant zinc deficient women or in experimentally induced dietary deficiency.

In summary, these data indicate that pregnant monkeys fed a marginally zinc-deficient diet show zinc deficiency signs during the 3rd trimester. In addition, despite otherwise optimal dietary and environmental conditions, they demonstrate a compromised physiological condition as reflected in inadequate weight gain, reduced circulating glucose, triglycerides and vitamin A, slight iron deficiency anemia, and reduced immune response. Such pregnancies can be considered nutritionally at risk for poor fetal outcome.

The authors thank Wil Saito for his major role in data collection and immunoassays; Peter Takeuchi for technical assistance; Dr T Tamura for folate assays and Dr R Robbins for complement assays; and also the following student assistants: Darrell Haynes, Wanda Arrandell, Mary Kesler, Rebecca Pierce, Art Rodriguez, Kerry Gott, Kris Ivani, Tammy Hendrie, Terry Vaso, and Lois Suzuki.
REFERENCES

39. Berchelmann ML, Vice TE, Kalter SS. Peripheral blood changes in the pregnant (Kenya) baboon