Use of the intramuscular relative-dose-response test to predict bronchopulmonary dysplasia in premature infants

Richard D Zachman, David P Samuels, Joe M Brand, Jim F Winston, and Ju-Tsung Pi

ABSTRACT  We tested the hypothesis that an intramuscular relative dose response (IM-RDR) on day 1 of life would more accurately predict which premature infants would develop bronchopulmonary dysplasia (BPD) than single measurements of retinol, retinyl palmitate (RP), or retinol binding protein (RBP). Seventy-five premature infants ≤ 32 wk gestation had the IM-RDR on day 1 of life. An RDR ≥ 25% occurred in 6 of 37 infants who did not develop BPD compared with 15 of 38 infants who developed BPD (p = 0.025). Retinol, RP, and RBP on day 1 were not different between the groups. BPD infants who received postnatal dexamethasone during their hospital course had a higher day-28 baseline retinol concentration (1.19 ± 0.15 μmol/L) than did the group with no BPD (0.82 ± 0.06 μmol/L) (p = 0.03). However, the effect of postnatal dexamethasone on serum retinol was bimodal, rising initially, and then declining after 8–12 d. RP values at time 0 and 5 h on day 28 were higher than day 1 values in both infants without BPD and infants with BPD who did not receive dexamethasone. Retrospective analysis also revealed a significant correlation between a day-1 RDR ≥ 25% and the incidence of intraventricular hemorrhage in premature infants. Because the IM-RDR is more helpful in predicting the development of BPD than single serum retinol and RP analyses, this test could be useful in determining which premature infants might benefit from supplemental vitamin A for BPD.  Am J Clin Nutr 1996;63:123–9.

KEY WORDS  Vitamin A, relative dose response, bronchopulmonary dysplasia, premature infant, retinol, retinyl palmitate, retinol binding protein, dexamethasone, intraventricular hemorrhage

INTRODUCTION

Despite years of observations, the indicators of physiologically adequate vitamin A in premature human infants in relation to prevention and healing of premature infant lung disease are not clearly defined. Bronchopulmonary dysplasia (BPD) is a chronic lung injury disease that often results from ventilator and oxygen therapy used to treat neonatal respiratory distress. The incidence of BPD is highest in premature infants (1). Therapy strategies include mechanical ventilation, supplemental oxygen, fluid management, diuretics, bronchodilators, corticosteroids, and nutritional intervention. Despite these therapies, BPD is a serious disease with considerable morbidity (1–4); thousands of infants are affected each year in the United States alone (1).

Vitamin A is necessary for normal lung growth and cellular activity (5) and it has been suggested that vitamin A plays a role in the prevention or resolution, or both, of lung injury in BPD. Observations that support this rationale are the following. 1) Vitamin A deficiency results in squamous metaplasia of columnar tracheal epithelium with the loss of cells that produce cilia and mucus, necrotizing bronchiolitis, and other histopathology similar to BPD (4–9). 2) Low liver retinyl ester stores have been demonstrated after death in premature infants weighing < 1500 g (10). 3) Many reports show that plasma retinol concentrations in premature infants < 36 wk gestation are lower than those in term infants (5, 11, 12). Some data suggest that premature infants developing BPD have lower initial plasma retinol concentrations than do those who do not develop BPD, but this is not a consistent finding (12–16). Finally, some clinical trials in which supplemental vitamin A was used reported a decrease in the incidence of BPD and its associated morbidity in premature infants (15, 17, 18–20). However, these observations also remain controversial (21) and such supplements are not in common use. Even in studies in which supplemental vitamin A was used, 30–50% of those patients treated still developed BPD (15, 18, 21).

Several factors potentially interfere with the clarification of the relationship between vitamin A and BPD: increased use of antenatal and postnatal steroids that affect vitamin A metabolism (11, 22–24), changed criteria for the diagnosis of BPD (1), and an unknown lower limit for the concentration of tissue vitamin A that might cause functional biochemical deficits in premature infant lung. Finally, all conclusions of previous studies were based on a single plasma retinol value, which, unfortunately, does not correlate well with liver stores until it becomes very low (< 0.35 μmol/L (< 10.0 μg/dL)) (10, 11, 25–27), and in some populations even lower (< 0.17 μmol/L (< 5 μg/dL)) (28). Many authors note this problem (5, 10, 13, 18, 25, 27) but the use of a single retinol concentration continues in the evaluation of premature infants.

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3 Address reprint requests to RD Zachman, University of Wisconsin, The Center for Perinatal Care, 202 South Park Street, Madison, WI 53715. Received March 15, 1995. Accepted for publication September 21, 1995.
The relative dose response (RDR) (29) and the recently described didehydroretinol response (modified RDR) (30–32) more accurately reflect vitamin A stores. In the RDR method, serum retinol is measured at time 0, an oral retinyl ester dose is administered, and serum retinol is measured again 5 h after the dose. The difference between the two serum retinol measurements reflects stores of vitamin A in the liver. A high percentage change of retinol from time 0 to 5 h after dosing (high RDR%) reflects low liver vitamin A stores and vice versa. The test was first validated with measurements of actual rat liver vitamin A (29) and has since been correlated with liver vitamin A stores determined by needle biopsy (33) and postmortem liver analysis in human adults (34). Vitamin A–deficient children with and without viral infection, children with acute diarrhea, and term newborns had decreasing RDR values after a large supplemental vitamin A dose was given, suggesting improved tissue stores of vitamin A (35–37). The oral RDR has been used previously in 83 preterm infants shortly before they were discharged (38). The regression of comparison of the RDR to retinol was −0.58. Three-fourths of these infants had an RDR > 20%, suggesting that even relatively healthy preterm infants had suboptimal vitamin A status at discharge.

Our primary objective was to test the hypothesis that an intramuscular relative dose response (IM-RDR) on day 1 of life would be of more value in predicting which premature infants would develop BPD than single measurements of serum retinol, retinyl palmitate (RP), or retinol binding protein (RBP). Based on the IM-RDR method recently established in rats (39), our specific prospective hypothesis was that an RDR $\geq 25\%$ (low vitamin A stores) on day 1 would be significantly correlated with the development of BPD. A secondary objective was to determine whether vitamin A assessments on day 28 of life were different in premature infants developing BPD compared with those without BPD. Third, we report data confirming the work of others that postnatal steroid use alters serum retinol concentrations in human neonates. Finally, we found retrospectively that an IM-RDR $\geq 25\%$ was significantly associated with intraventricular hemorrhage (IVH) in these premature infants.

**SUBJECTS AND METHODS**

**Subjects**

During 1990–1993, 110 premature infants $\leq 32$ wk gestation were prospectively enrolled into the study in their first 24 h of life. Thirty-five patients were excluded for various reasons (Table 1). Patients were enrolled under the identical protocol in two State of Wisconsin Perinatal Centers (University of Wisconsin, Meriter Hospital, Madison, and St Vincent’s Hospital, Green Bay). The protocol was approved by the University of Wisconsin Human Subjects Committee and the Meriter Hospital and Green Bay Hospital Institutional Review Boards.

An initial 0.8-mL blood sample was drawn within 24 h after birth before any vitamins or nutrition was given. The infant then received an intramuscular dose of RP (5000 IU/kg, ie, 1500 $\mu$g = 2.85 $\mu$mol; Aquasol A, water-miscible vitamin A palmitate, Armour Pharmaceutical Company, Kankakee, IL). Five hours later, another 0.8-mL blood sample was drawn. The sera were each isolated shortly after sampling and stored at −20 °C until analyzed.

The infants’ gestational ages were determined by early obstetrics ultrasound dating, or after birth by physical exam criteria (40). There were no gestational age discrepancies in the patients whose data are analyzed here. The criteria for the diagnosis of BPD included a history of respiratory distress syndrome, X-ray findings of BPD (41), and a requirement for supplemental oxygen at 36 wk postconceptional age (1). Additionally, the diagnosis of BPD was assigned if patients had received steroids at $\geq 6$ d of life to facilitate weaning from the ventilator, or had pulmonary findings at autopsy such as interstitial emphysema or pneumothorax, interstitial fibrosis, and histology compatible with BPD (9). In the 75 singleton patients entered into the study (39 $\leq 28$ wk, with mean birth weight = 866 $\pm$ 164 g; 21 = 29–30 wk, with mean birth weight = 1356 $\pm$ 197 g; and 15 = 31–32 wk, with mean birth weight > 1590 $\pm$ 249 g), the incidence of respiratory distress syndrome was 97.4%, 76.2%, and 40% and the incidence of BPD was 82%, 19%, and 13% in the three groups, respectively. Forty-seven of the 63 patients with respiratory distress syndrome received exogenous surfactant (1–3 doses) in rescue protocols.

After the RDR was completed on day 1, infant nutrition was first given by intravenous hyperalimentation and then later by enteral methods when tolerated. The infants were given an average daily amount of vitamin A of 840–1000 IU (250–300 $\mu$g/kg) for the first 28 d. An RDR was repeated again at day 28 in 53 of the original 75 patients that were evaluated on day 1. The reasons for the loss of the 22 patients by day 28 were lack of data because of inadequate sample or patient transfer (16) and BPD death before 28 d (6) (Table 1). In addition, sufficient serum was not always available for all the analyses planned.

Postnatal dexamethasone treatment for BPD was begun in 18 patients that completed the study. In three of these patients, baseline retinol concentrations were measured just before dexamethasone treatment began and again 24 and 48 h after the start of treatment. In the 15 remaining patients, a single 28-d serum retinol value was measured, 8–22 d after the steroid treatment.

### Table 1

<table>
<thead>
<tr>
<th>Patient population analyzed$^1$</th>
<th>Day 1</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients aged $\leq 32$ wk</td>
<td>110</td>
<td>75</td>
</tr>
<tr>
<td>Excluded from RDR analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate retinol data, discharge, or transfer of patients to referring hospital</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Intrauterine growth retardation$^2$</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Early deaths ($&lt;5$ d) due to pulmonary hemorrhage, hypoplastic lung, or seizures</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Discordant twin sets (1) and triplets sets (2)$^2$</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Antenatal maternal steroids</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>BPD deaths (days 6–28)</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>No BPD at 36 wk</td>
<td>37</td>
<td>28</td>
</tr>
<tr>
<td>BPD diagnosis at 36 wk</td>
<td>38</td>
<td>—</td>
</tr>
<tr>
<td>No postnatal steroids</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Received postnatal steroids</td>
<td>18</td>
<td>—</td>
</tr>
</tbody>
</table>

$^1$ RDR, relative dose response; BPD, bronchopulmonary dysplasia.
$^2$ $< 25\%$ percentile birth weight.
$^3$ $> 10\%$ birth weight differential.
was started (i.e., in patients who received dexamethasone on day 6, 22 d elapsed before the measurement was taken).

Analysis of retinol, retinyl palmitate, and the RDR

HPLC was used for the analysis of serum retinol and RP as described previously (39). In brief, duplicate 0.1-mL samples of serum were extracted with one volume of 100% ethanol and two volumes of hexane containing 0.5 g butyalted hydroxytoluene/L. The hexane layer was removed and the aqueous phase was extracted three more times. The combined hexane extracts were evaporated under nitrogen at 30 °C in the dark. The dried extract was solubilized in 30-50 μL methanol: chloroform (4:1) and kept on ice in the dark under nitrogen until aliquots were taken for HPLC analysis.

A Perkin-Elmer (Norwalk, CT) HPLC system was used. The isocratic mobile phase was methanol:chloroform:water (80:15.5: by vol) at a flow rate of 1.5 mL/min, and the detection wave length was 325 nm. The retention times were 1.53-1.56 min for retinol and 4.40-4.46 min for RP. With each series of sample analysis, 7-10 different amounts of standards of commercially available all-trans-retinol and RP (Sigma Chemical Company, St Louis) were used as quantitative references. The square of the correlation coefficient (r²) for these standard curve analyses was 99-100%. Addition of retinyl acetate as an internal standard before extraction indicated repeatedly that the extraction procedure was 98-100% efficient. The RDR was calculated as previously described (29, 39):

\[ RDR\% = \frac{(A_s - A_0)}{A_s} \times 100 \]

where \( A_0 \) and \( A_s \) are the serum retinol concentrations at time 0 and 5 h, respectively. On the basis of previous work with the IM-RDR in rats, we prospectively selected an RDR ≥ 25% as the cutoff to represent low liver reserves of RP. When enough serum was available, RBP was measured by a quantitative radial immunodiffusion method [Human Radial Immunodiffusion Kits (806001), Behring Diagnostics, Summerville, NJ].

Statistical analysis

Statistical analyses were performed using SAS version 6.09 (SAS Institute Inc, Cary, NC). Chi-square tests were used to analyze categorical data if the size of each cell was large enough; otherwise, the Fisher exact test was used. Differences between groups in continuous responses were tested using \( t \) tests. Some healthier patients were discharged or transferred, some died before day 28, and some were inadequately sampled; thus, there were fewer patients at day 28 than day 1. Although analysis of variance might detect certain interactions among days and groups, we avoided biasing comparisons of day 1 and day 28 by performing paired \( t \) tests for between-day comparisons. Analysis of covariance was used to assess the relation for both categorical and continuous independent variables with continuous dependent variables with continuous responses. For binary dependent variables, logistic regression was used. Pearson correlation coefficients were calculated to describe the strength of relation among continuous variables. Unless otherwise specified, test of hypothesis used an overall significance level of 0.05.

RESULTS

Analyses at day 1

An IM-RDR ≥ 25% during the first 24 h of life was significantly associated (\( P = 0.025 \)) with the development of BPD (Table 2). No other determination of vitamin A status had any predictive association with BPD (Table 2). Day 1 mean serum values of retinol at time 0 were not significantly different. The number of premature infants with retinol concentrations < 0.525 μmol/L (< 15 μg/dL) or < 0.70 μmol/L (< 20 μg/dL) were not significantly different between those that developed BPD and those without BPD (Table 2). Also, the number of premature infants with retinol concentrations < 0.35 μmol/L (< 10 μg/dL) were not different (data not shown). The analyses of the number of premature infants with retinol concentrations < 0.525 μmol/L or < 0.70 μmol/L were by a two-by-two table created for each of these cutoffs. In each case there was no significance by a chi-square test, indicating that none of the chosen retinol concentrations were related to the development of BPD. Likewise, there was no statistically significant difference in the day-1, baseline mean RBP or RP concentrations at time 0.

As expected, and noted above, 82% of infants ≤ 28 wk gestation developed BPD compared with 16.7% at 29-32 wk (\( P < 0.001 \)). The incidence of BPD was also significantly inversely correlated with birth weight. The frequency of RDR ≥ 25% tended to be higher in the lowest gestational age group (< 28 wk) but the difference was not significant (\( P = 0.11 \)). Covariance analysis using either gestational age or birth weight with an RDR ≥ 25% did not improve the prediction of the development of BPD. Additional retrospective analyses showed there was no statistically significant association of RDR ≤ 15%, or ≤ 20%, with the incidence of BPD. Forty-four percent of patients had an RDR > 15 and 37% had a RDR > 20.

Analyses at day 28

Three groups were analyzed at day 28: patients without BPD (No BPD); patients with BPD but not treated with postnatal dexamethasone (BPD–No DEX); and patients with BPD and treated with postnatal dexamethasone (BPD-DEX) (Table 3). Paired analyses were done on these three groups.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Day 1 vitamin A assessment in predicting the development of bronchopulmonary dysplasia (BPD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Assessment</td>
<td>No BPD</td>
</tr>
<tr>
<td>RDR ≥25% / 1</td>
<td>6/37</td>
</tr>
<tr>
<td>Retinol (μmol/L)</td>
<td>0.70 ± 0.035 [37] / 1</td>
</tr>
<tr>
<td>Number of patients with retinol ≥0.70 μmol/L</td>
<td>18</td>
</tr>
<tr>
<td>Number of patients with retinol ≥0.525 but &lt;0.70 μmol/L</td>
<td>13</td>
</tr>
<tr>
<td>Number of patients with retinol &lt;0.525 μmol/L</td>
<td>6</td>
</tr>
<tr>
<td>Retinyl palmitate (μmol/L)</td>
<td>0.045 ± 0.015 [29]</td>
</tr>
<tr>
<td>Retinol binding protein (mg/L)</td>
<td>18 ± 1.0 [33]</td>
</tr>
</tbody>
</table>

1 Relative dose response.
2 Significantly different from No BPD group, \( P = 0.025 \).
3 \( \bar{x} \) ± SEM at time 0; \( n \) in brackets.

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There was no statistically significant difference in the percentage of patients with RDR ≥ 25% on day 28 between the groups (No BPD, 6/28; BPD–No DEX, 4/7; BPD-DEX, 6/18). There was no difference in the mean retinol concentration at time 0 in the No BPD group compared with the BPD–No DEX group. However, patients who received postnatal dexamethasone (the BPD-DEX group) had a higher day-28 mean retinol concentration at time 0 compared with the No BPD group (P = 0.03, using the two-sample t test; Table 3). The BPD-DEX group also had a significant difference in their retinol concentrations at time 0 between day 1 and day 28 (Table 3).

Day 28 RP concentrations at time 0 were variable and not different between the three patient groups. However, paired t test analysis (Table 3) showed significant differences between day 1 and day 28 in the No BPD group (P < 0.001) and in the few patients in the BPD–No DEX group (P = 0.05). The 5-h RP concentrations were overall roughly twice as high on day 28 as they were on day 1, and were significantly higher at day 28 than at day 1 in the No BPD and BPD–No DEX groups (Table 3).

RBP time 0 concentrations were not different at day 28 between the three groups of patients (Table 3). However, 5-h RBP concentrations in the BPD–No DEX and the BPD-DEX groups at day 28 were higher than the corresponding 5-h values on day 1 (Table 3). This difference did not occur in the No BPD group. The day-28 RBP value was also evaluated as the percentage change between samples at time 0 and 5 h in each patient and means in each group were calculated (18). Percentage change was highest in the BPD–No DEX patient group (63.2%), and this value was significantly different from that in No BPD group (10.7%) (P < 0.05). Percentage change in the BPD-DEX group was 14.5%, but this was not different from either of the other two groups.

Postnatal dexamethasone treatment caused an initial rise in serum retinol concentrations (Figure 1). At 8–12 d after starting dexamethasone, there was an apparent decrease in serum retinol during the time it was measured.

The IM-RDR and intraventricular hemorrhage

Although not part of the original objectives, the data show that an IM-RDR ≥ 25% was significantly associated with both total and severe (grade 3–4) IVH. When the RDR was ≥ 25%, the incidence of grade 3–4 IVH was 42%, but in those patients with RDR < 25%, the incidence of grade 3–4 IVH was only 6% (P = 0.005) (Figure 2). However, mean concentrations of retinol, RBP, or RP or frequency of low retinol concentrations (< 0.525 μmol/L or < 0.7 μmol/L) were not significantly related to the occurrence of IVH (data not shown).

DISCUSSION

An IM-RDR at day 1 was used here to determine the association of the vitamin A status of premature infants with their chances of developing BPD. The validity of the IM-RDR was not proven by direct correlation with human premature liver samples because present-day human research protocol constraints and lower mortality and autopsy rates in neonatal intensive care patients precluded this. However, several observations support the reliability of the IM-RDR method, and thus, the rationale for its use in this study. First, an oral RDR on the first day of life in sick premature infants is not practical, because most receive only intravenous fluids for the first few days of life. Second, the IM-RDR test is simple and easy to use. Finally, previous studies have shown that the IM-RDR test provides reasonably accurate results, and the IM-RDR test has been used in other studies to determine circulating vitamin A status in premature infants.
VITAMIN A AND BPD

FIGURE 2. Relation between day-1 intramuscular relative dose response (IM-RDR) ≥ 25% and the incidence of grade 3–4 intraventricular hemorrhage (IVH). The day-1 IM-RDR was calculated as described in the text. IVH was diagnosed by cranial ultrasound, and grades 3–4 were diagnosed by the criteria of Papille et al. (42).

days of life. An intramuscular IM-RDR method was therefore developed and rigorously tested in young adult rats, in which an RDR > 25% consistently reflected low RP stores in both liver (39) and lung (RD Zachman, unpublished data, 1993). Second, the IM-RDR has been previously used by others on human premature infants and data reported in abstract form (43–45). The optimal blood-sampling time was established as 5 h after the vitamin A dose (43), the same as in the rat model (39). In another study, the IM-RDR was used to measure a response of a supplemental vitamin A dose (44), and a third study showed a worsening (rising RDR%) at day 28 in non-supplemented premature infants, whereas those supplemented with vitamin A maintained an RDR% < 30% (45). Last, we did have factual correlation for one patient who died 6 h after the IM-RDR as the result of complications of immaturity. In this patient, the RDR was 17% and the liver biopsy RP was 0.29 μmol/g, representing a good tissue storage concentration of vitamin A.

The IM-RDR of ≥ 25% on day 1 in premature infants was statistically correlated with the development of BPD. The correlation could not be improved by combining the IM-RDR with gestational age, trying to separate out mild or severe BPD (data not shown), or by retrospectively using other RDR% cutoffs. No other vitamin A assessment made, including mean retinol, RP, and RBP concentrations or number of infants with retinol concentrations < 0.70 μmol/L or ≤ 0.525 μmol/L, predicted the development of BPD. The multiplicity of t tests in these analyses could be a problem because by chance alone 1 of 20 comparisons might be statistically significant. However, the significant difference found was with the IM-RDR ≥ 25%, which was predicted in our hypothesis based on the previous animal experiments (39). Even this was, disappointingly, not an improvement on the already well-established relation between low gestational age, low birth weight, and incidence of BPD.

Serum RP in premature infants has not been reported previously. RP in premature serum on day 1 was roughly 7% of the serum retinol concentration. Concentrations of RP at time 0 on day 28 were 20–25% of the serum retinol concentration, and significantly higher than on day 1 in patients who had not received dexamethasone. This increase in RP at time 0 on day 28 might indicate improved vitamin A storage in these premature infants. Changes in the baseline concentration of RP have been correlated with supplemental vitamin A intake in older subjects (46). The twofold higher RP at 5 h on day 28 might also represent a better overall storage of vitamin A, with less immediate tissue uptake of the intramuscular RP dose. However, 25% of the premature infants without BPD and 40% of those with BPD still had an IM-RDR ≥ 25% on day 28, and there was no correlation between an IM-RDR ≥ 25% and RP concentrations at either day 1 or day 28. These observations do not support the above suggestion that higher serum RP concentrations at day 28 indicate better vitamin A stores. The findings here at day 28 are similar to those of Woodruff et al. (38), showing that many premature infants apparently are not receiving enough vitamin A to alter their serum or storage vitamin A concentrations. Supplements are probably needed in many premature infants but a major question is still what the ideal concentration is to strive for during the first weeks.

A maturational change in absorption or clearance of the RP dose could also explain the differences in the day-1 and day-28 baseline RP and 5-h response concentrations. Although these observations are original, their meaning will require further study.

Shenai et al. (18, 19) proposed that assessing the percentage change in RBP concentrations from 0 to 6 h at 28 d is a reliable method to study the usefulness of supplemental vitamin A in BPD. Our findings, albeit on a small number of patients, support the proposal that patients with BPD have a larger percentage change in RBP from 0 to 5 h on day 28 than do those without BPD.

Antenatal maternal glucocorticoids enhance fetal lung maturity and lower the incidence of neonatal respiratory distress syndrome and its associated morbidities (11, 47). Because glucocorticoids affect vitamin A metabolism and concentrations (11, 22–24), we did not enroll neonates who were born of mothers being treated with antenatal steroids. The remarkable fact that only 20% of the mothers of the premature pregnancies screened received antenatal steroids is consistent with recent use in the United States (48, 49). Dexamethasone is also used in the postnatal treatment of BPD (1, 50, 51) and therefore the 28-d analyses reported here compared patients with no BPD to patients with BPD but who were not treated with dexamethasone and with patients with BPD who were treated with dexamethasone. A previous report showed that serum retinol, RBP, and transthyretin were elevated for 3–14 d after the start of dexamethasone treatment (23). Our data suggest a similar time course of neonatal serum retinol after dexamethasone treatment (Figure 1). Serum retinol increased for at least the first 48 h after steroid administration, suggesting that dexamethasone mobilized liver stores of retinol to the serum, or suppressed tissue uptake. After 10–12 d, concentrations of retinol decreased again. Although these numbers were obtained from separate patients, the trend is exactly the same as reported previously for serial samples (23). This subsequent decrease could be in response to decreases in the dexamethasone treatment dose. In our patients, the dexamethasone dose was decreased one-half or one-third every 3 d of therapy (4, 50, 51). On the other hand, the fall in serum retinol could be the result of exhaustion of liver vitamin A stores, which is then reflected in the decreasing serum retinol concentrations. Postnatal dexamethasone treatment of neonatal rat pups does actually deplete
both liver and lung stores of retinol and RP (24). This hypothesis is not supported with the human RDR data presented here because there was no difference in the number of patients with an RDR $\geq 25\%$ in the BPD-DEX group (6/18) and the BPD-No DEX group (4/7) at day 28. An association might have been missed because the numbers in each group are relatively small.

The final observation of potential clinical importance made here was that an IM-RDR $\geq 25\%$ on day 1 was associated with a higher percentage of premature infants with IVH than an IM-RDR $< 25\%$. The relation was noted with all IVH grades, especially in the clinically important group of grade 3–4 IVH that is accompanied by significant morbidity (42, 52). This study was not designed prospectively to assess the IVH question, so we cannot prove that the patient group with an IM-RDR $\geq 25\%$ and that with an IM-RDR $< 25\%$ were comparable for all factors that might contribute to the incidence of IVH. The incidence of pneumothorax, hypotension, degree of acidosis, asphyxia, and other factors that might contribute to IVH were not reviewed retrospectively. However, all the patients did have cranial ultrasounds: the gestational age of the group with an RDR $< 25\%$ and that with an RDR $\geq 25\%$ was the same and the distribution of patients at each gestational age was similar in the groups both with and without IVH. In agreement with our observation, one previous clinical study of postnatal vitamin A supplementation showed that the incidence of the more severe grades of IVH was lower with vitamin A supplementation (53). Vitamin A might prevent IVH by either free radical scavenging or by maintaining the integrity and regeneration of the supporting germinal matrix. Further research in this area would be of interest.

In summary, this study shows that single day-1 serum retinol, RP, or RBP concentrations are poor predictors of the subsequent development of BPD in premature infants born $\leq 32$ wk gestation. An IM-RDR $\geq 25\%$ more accurately predicts the chances of having BPD and is also more predictive of IVH than any other of the serum vitamin A assessments made. If supplemental vitamin A is being considered for the prevention or treatment of BPD, we suggest that screening for those patients who might benefit from supplemental vitamin A is better done with an IM-RDR. The obvious disadvantages of the IM-RDR are the need for an injection and two serum retinol samples. A less invasive method of tissue retinoid assessment, such as the modified RDR (31, 32), might be more useful in this premature population if parenteral administration could be standardized; use of the modified RDR in premature infants should be studied. Because dexamethasone is frequently used in BPD patients in many neonatal intensive care programs, its effect on premature infant serum and tissue vitamin A needs further study.