Pediatr. Res. *16:* 75–78 (1982)

Early Oral Administration of Vitamin D and its Metabolites in Premature Neonates. Effect on Mineral Homeostasis

BERNARD L. SALLE,[32] LOUIS DAVID, F. H. GLORIEUX, E. DELVIN, JACQUES SENTERRE, AND HUBERT RENAUD

Neonatal Department, Hôpital Edouard-Herriot, 69374 Lyon Cedex 3, Unité INSERM U.34, Hôpital Debrousse 69005 Lyon, France, and Genetics Unit, Shriners Hospital, Mc Gill University, Montreal, Canada

Summary

For five days, three groups of six premature infants each were fed human milk and given a daily dosage of one of the following: vitamin D3 (30 μg), 25-OH D3 (10 μg) and 1,25-OH D3 (0.5 μg). The infants in the groups were matched for gestational age and birthweight. Administration of 25-OH D3 or 1,25-$(OH)_2$ D3 did not significantly modify the course of early neonatal hypocalcemia as compared with infants receiving vitamin D3. Mean plasma Ca ± S.D. (mg/100 ml) decreased to nadir values at 48 hr (D3: 5.7 ± 1.2; 25 OH D3: 6.8 ± 0.9; 1,25-$(OH)_2$ D3: 6.7 ± 1.1). A progressive increase toward normal values was seen at 120 and 168 hr in the three groups. Mean plasma immunoreactive parathyroid hormone ± S.D. (μl Eq/ml) followed an opposite pattern with peak values at 48 hr (D3: 231 ± 137; 25-OH D3: 281 ± 138; 1,25-$(OH)_2$ D3: 211 ± 149). Mean plasma ± S.D. 25-OH D values (ng/ml) were low at 1.2 hr (8.7 ± 4.8) n: 16) and increased significantly after 7 days of D3 (18.2 ± 4.2 $P < 0.001$) and 25-OH D3 administration (46 ± 10.3 $P < 0.001$)/ Mean plasma iCT ± S.D. (pg/ml) reached peak values at 24 hr (D3: 457 ± 186; 25-OH D3: 415 ± 121; 1,25-$(OH)_2$ D3: 443 ± 183). These data suggest that the various forms of vitamin D are well absorbed in preterm infants and that administration of vitamin D metabolites during the first days of life is not warranted for the prophylaxis of early neonatal hypocalcemia.

Speculation

A defect in vitamin D metabolism is considered to be a possible pathogenetic factor in early hypocalcemia in premature infants. Since our data indicate adequate intestinal absorption and liver hydroxylation of vitamin D3 with no obvious effect on the D metabolites, it is speculated that the biotransformation pathway of vitamin D3 is operative in preterm infants after 32 wk of gestation.

Premature neonates present during the first 12 to 24 hours of life a decrease in plasma calcium (Ca) levels which leads frequently to neonatal hypocalcemia (7, 9, 20, 22, 26). We have shown previously that this is apparently not the result of impaired parathyroid function because most infants demonstrate a rapid increase in plasma immunoreactive parathyroid hormone (iPTH) levels in response to falling plasma Ca levels (1, 2, 9, 20). In addition, there is supporting evidence that hypercalcitoninemia may contribute to this early disturbance of calcium metabolism (2, 4, 8, 10, 20).

Oral administration of 25 hydroxycholecalciferol (25-OH) or 1,25-dihydroxycholecalciferol [1,25-$(OH)_2$ D3] appeared to improve plasma Ca concentration in premature infants (5, 13) and led to the hypothesis that vitamin D biotransformation was not adequate in these prematures (18, 19).

The present study compares the effects of oral administration of vitamin D3, 25-OH D3 and 1,25-$(OH)_2$ D3 and mineral homeostasis in premature infants during the first week of life. Our results suggests that neither 25-OH D3 nor 1,25-$(OH)_2$ D3 deficiency is likely to be a significant factor in the pathogenesis of early neonatal hypocalcemia.

INFANTS AND STUDY DESIGN

The study involved 18 premature infants who were transferred during the first hour of life to the newborn intensive care unit of the Edouard Herriot Hospital, Lyon, France. The gestational age was assessed by the Dubowitz scoring system (11). Informed consent was obtained from the parents.

The subjects were randomly divided into three groups. The first group was given a daily oral dose of 30 μg (1 200 IU) of vitamin D3. The second group received 10 μg of 25-OH D3 daily and the third a daily dose of 0.5 μg of 1,25-$(OH)_2$ D3 (supplied by Hoffman Laroche Laboratories, Basel). All vitamins were mixed with 3 ml of human milk and administered by tube feeding.

Mean birth weight and gestational age were similar among the three groups (Table 1). All infants had a birth weight below 2500 g and one was small for gestational age. There was no history of maternal diabetes or infections in any of them. Blood pH was determined in all instances just after birth and later checked regularly (24).

All infants were continuously infused with a 10% glucose solution (80 ml/kg/day) without calcium using a Braun Melsungen pump. The infusion was started between 1 and 2 hr of age after the first blood sampling and maintained for 5 days. Human milk was commenced at 12 hr of age; because the range of their birth weight was relatively small (1900–2400 g) all infants received the same amount of milk during the time of the study. The amount of Ca and phosphorus (P) to be received from birth to the seventh day of life was calculated according to Barltrop and Hillier (3) (Table 2). In each group the treatment was given as a single daily dose starting at 3 hr of age and then at 24, 48, 72, 96, and 120 hr.

Blood samples, 2- to 3-ml, were drawn by peripheral venous puncture using a scalp vein needle at 1 to 2, 24, 48, 120, and 168 hr. The samples were centrifuged immediately after collection and the plasma stored at −28°C until analysis. Furthermore during the fifth day of life, urine was collected from groups I and III during a 6-hr period (114 to 120 hr).

LABORATORY METHODS

Plasma Ca and P (14, 15) were determined colorimetrically on an automatic Technicon SMA 12/60 analyzer (R) modified for microvolume analysis. Plasma iPTH levels were determined by radioimmunoassay as described previously (6, 7). Plasma iPTH levels in normal children and adults ranged from nondetectable

Table 1. *Clinical data of the three groups of infants studied*

	Premature infants with oral vitamin D3	Premature infants with oral 25 OH D3	Premature infants with oral 1,15-$(OH)_2$ D3
No. of infants	6	6	6
Birth weight (g)			
Mean and ± S.D.	2091 ± 96	2126 ± 252	2168 ± 160
Range	1980–2200	1820–2460	1900–2300
Gestational age (wk)			
Mean and ± S.D.	34.8 ± 1.6	34 ± 2.2	34.6 ± 1.5
Range	32–36	32–35	32–36
pH at birth			
Mean and ± S.D.	7.34 ± 0.06	7.31 ± 0.03	7.31 ± 0.02

Table 2. *Estimation of daily intake of calcium and phosphorus in mg/day calculated from Baltrop and Hillier (3)*

Days	1	2	3	4	5	6	7
Total amount of calcium given by human milk (mg/day)	4.9	19.6	29.4	39.2	49	58.8	68.6
Total amount of phosphorus given by human milk (mg/day)	2.7	10.8	16.2	21.6	27.2	32.4	37.8

(i.e., < 25 μl Eq/ml) to 100 ml Eq/ml with 95% of detectable values. Plasma immunoreactive calcitonin (iCT) levels were also determined by radioimmunoassay (25). In our laboratory, levels are undetectable in normal children and adults while very high values are consistently found in patients with medullary carcinoma of the thyroid. To measure 25-OH D and 1α,25-$(OH)_2$, the tracers 25-hydroxy(26,27-^{3}H-methyl)cholecalciferol and 1α,25-dihydroxy [23,24-(n)-^{3}H]cholecalciferol (Amersham-Searle, Oakville, Ont., Canada) were added to serum. The mixture was extracted and chromatographed to separate the vitamin D metabolites (12). The fraction containing 25-OH D was assayed in triplicate according to the method of Haddad and Chyu (17). The mean concentration (±S.D.) and range observed in samples collected from 40 healthy infants and children (8 to 36 months of age) from November to April are 20.2 ± 4.3 and 13.9 ± 38.1 ng/ml, respectively. Urinary cyclic adenosine monophosphate (cAMP) was measured by the method of Walton and Garren (27) and hydroxyproline by the method of Prockop and Udenfriend (21).

CALCULATION AND STATISTICAL ANALYSIS

For each period, results were expressed as mean ± S.D. Samples containing no detectable iCT or iPTH were arbitrarily assigned a value corresponding to half the limit of detection of the assays, ie 75 pg/ml for iCT and 12.5. μl Eq/ml for iPTH.

Statistical analyses included paired *t* test, Student's *t* test, and regression analysis.

RESULTS

CALCIUM

The pattern of changes was similar in the three groups of infants: from moderately reduced values at 1–2 hr, mean levels reached a nadir at 24 hour ($P < 0.01$). From 24 to 48 hr, the changes were not significant: small increase in group II (+0.3 mg/100 ml), small decrease in groups I and III (−0.5 and −0.2 mg/100 ml, respectively). Analysis of the individual values indicated that three infants in group III and four infants in groups I and II had plasma Ca levels below 7 mg/dl at 24 and/or 48 hr. After 48 hr, all groups showed significant increases. At 168 hr, values were slightly above the normal range in groups I and III and within the normal range in groups II (>8.5 mg/100 ml).

Table 3. *Mean plasma levels of calcium (mg/100 ml), phosphorus (mg/100 ml), iPTH (μl Eq/ml), and iCT (pg/ml) during the first 7 days of life in the three groups studied (see details in the text)*

	1–2 hr			24 hr			48 hr			120 hr			168 hr		
Groups	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
No. of patients	6	6	5	6	6	6	6	6	6	6	6	6	6	5	5
Serum Ca (mg/100 ml)															
Mean	8.4	8.4	8.7	6.4[1]	6.6[1]	7[2]	5.8[1]	6.8[1]	6.7[1]	7.4	8.1	8.1	8.5	9.0	8.6
S.D.	±0.8	±0.3	±0.7	±1.2	±1.0	±1.1	±1.2	±1	±1.1	±1.1	±0.7	±1.1	±0.8	±0.6	±1.1
Serum P (mg/100 ml)															
Mean	5.3	5.8	5.3	5.5	5.6	5.8	6	5.9	6.4	5.6	6.1	6.3[2]	5.6	5.6	6.3
S.D.	±0.9	±1.6	±0.4	±1.3	±1.0	±1.0	±1.5	±0.8	±1.2	±1.3	±0.9	±0.6	±1.0	±0.9	±0.9
Serum iPTH (μl Eq/ml)															
Mean	109	57	61	231	258[1]	203[1]	231	281[1]	211	222	204	127	200	132	90
S.D.	±150	±59	±76	±152	±145	±139	±137	±138	±145	±203	±183	±49	±218	±117	±61
Serum iCT (pg/ml)															
Mean	192	126	107	457[1]	415[1]	443[1]	317	277[1]	282[1]	121	158	121	110	123	90
S.D.	±158	±95	±49	±196	±122	±183	±183	±93	±149	±52	±46	±51	±55	±60	±38

[1] Means significant difference $P < 0.01$ from the values at 1–2 hr.
[2] Means significant difference $P < 0.05$ from the values at 1–2 hr.

The magnitude of decrease in the mean Ca level seemed greater in group I (−2.6 mg/100 ml) than in groups II and III (−1.6 and −2 mg/100 ml, respectively); however, this was not significant. Similarly, the magnitude of the subsequent increase, from 48 to 168 hr, was apparently smaller in groups II and III (+2.1 mg/100 ml) than in group I (+2.7 mg/100 ml) but the difference was again not significant.

PHOSPHORUS

There was a moderate but not significant increase in mean levels in groups I and II (Table 3). By contrast, infants receiving 1,25-$(OH)_2$ D3 had a significant increase at 120 hr ($P < 0.05$). Comparison among the three groups did not elicit any major differences at any time.

PARATHORMONE

The developmental patterns of mean iPTH were similar in the three groups with no significant differences. There was a clear-cut increase above normal values at 24 hr, little change from 24 to 48 hr and a decrease thereafter (Fig. 1; Table 3).

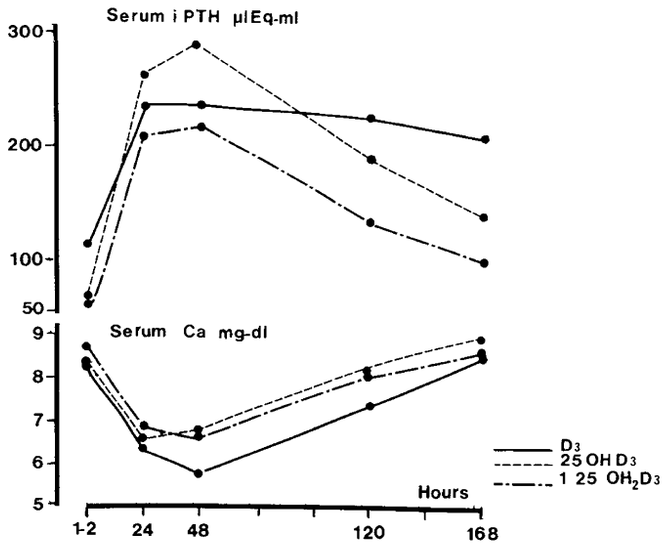

Fig. 1. Mean plasma calcium and iPTH levevls in the three groups during the first 7 days of life.

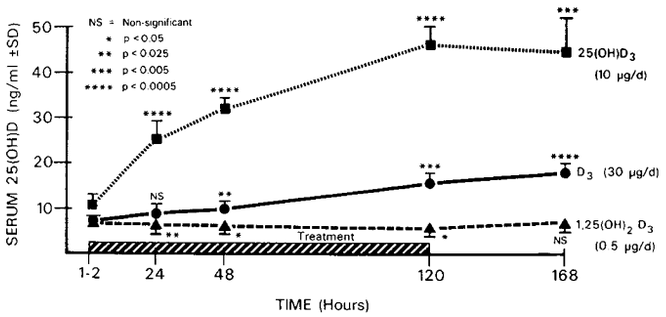

Fig. 2. Mean plasma 25 hydroxycholecalciferol in the three groups during the first 7 days of life.

CALCITONIN

Levels were low in all infants at 1–2 hr (Table 3). Four subjects in groups II and III and three in group I had nondetectable values (<150 pg/ml). There was a significant increase in mean values in the three groups at 24 hr ($P < 0.01$). This was followed by a decrease which was already manifest at 48 hr and even more evident at 120 and 168 hr. Four infants in groups I and III and three in group II had nondetectable levels at 168 hr. There were no significant differences among the three groups at any time.

SERUM 25-HYDROXYCHOLECALCIFEROL (FIG. 2)

The mean values at 1–2 hr were 8.7 ± 4.8 mg/ml (n 16). In group I, the values increased progressively during the entire period of study (18.2 mg/ml at 168 hr). In group II, there was a rapid rise which already had been significant at 24 hr ($P < 0.0005$). In group III, there was a significant decrease during the first 5 days of life ($P < 0.05$).

URINE STUDIES (TABLE 4)

Fractional excretion of Ca, P and cAMP on the 5th day were not different in groups I and III. By contrast the fractional excretion of hydroxyproline was significantly higher ($P < 0.05$) in group III, which received 1,25$(OH)_2$ D3, than in group I, which received vitamin D3.

DISCUSSION

In the present study, daily oral administration of D3, 25-(OH) D3, or 1,25-$(OH)_2$ D3 during the first 5 days of life did not significantly affect either the early decrease in plasma Ca concentration or the time required to return to normal values.

Daily supplementation of vitamin D (400 to 1 200 IU) in premature infants does not prevent early neonatal hypocalcemia (22). Furthermore the vitamin is adequately absorpted and hydroxylated in the liver after 32 wk of gestation (16). In contrast to recent proposals (13), we found that oral administration of 25-OH D3, which resulted after 168 hr in supraphysiologic levels of the compound, was inefficient in altering the course of plasma calcium changes during the first week of life.

Our findings are also at variance with those of Chan *et al.* (5) who reported that early daily administration of 1 µg of 1,25-$(OH)_2$ D3 resulted in a significant increase in plasma Ca levels and 0.05 $\mu g/kg^{-1}/day^{-1}$ had no effect. It is indeed likely that the daily oral dose of 0.5 mcg that we gave to our infants was well beyond their physiological requirement; therefore, the effect observed by Chan *et al.* on plasma Ca was pharmacologic.

It is noteworthy that owing to very low oral Ca intake during the first days of life, a stimulation of the intestinal Ca transport by 1,25-$(OH)_2$ D3 would probably have a limited effect on raising plasma Ca levels in premature infants. The positive results observed by Chan *et al.* (5) with a daily dose of 1 mcg are likely to a consequence of a direct mobilization of Ca from bone. This is substantiated by our findings of a higher urinary fractional excretion of hydroxyproline in the infants receiving 1,25-$(OH)_2$ D3 as compared with those receiving vitamin D.

Table 4. *Calcium, phosphorus, and hydroxyproline urinary excretion in groups I and III on the fifth day of life.*

	No. of infants studied	Calcium (mg/100 ml) / Creatinine (mg/100 ml)	Phosphorus (mg/100 ml) / Creatinine (mg/100 ml)	Hydroxyproline (mg/100 ml) / Creatinine (mg/100 ml)
Vitamin D3 group (mean and S.D.)	5	0.06 ± 0.06	0.47 ± 0.31	0.86 ± 0.21
1,25-$(OH)_2$ D3 group (mean and S.D.)	5	0.1 ± 0.1	0.74 ± 0.55	1.43 ± 0.51
Student's *t* test		NS[1]	NS	$P < 0.05$

[1] Not significant.

Our study indicates that in premature infants, whose gestational age is more than 32 wk, vitamin D is adequately absorbed in the gut and hydroxylated in the liver (16). Also 25-OH D3 is readily absorbed and the plasma level exceeds normal concentrations after two daily doses of 10 μg (mean increase 32.2 ± 5.7 ng/ml at 48 hr). The endogenous pool of vitamin D in human milk is insufficient to correct the low 25-OH D concentrations found during the first week of life in group III.

Serum P levels remained low in the three groups of infants as compared with infants fed modified cow's milk formula (26). There were minor increases throughout the study in the groups of infants receiving vitamin D3 or 25-OH D3 while infants receiving 1,25-$(OH)_2$ D3 demonstrated a significant increase at 120 hr. Chan *et al.* (5) made a similar observation in premature infants receiving a daily dose of 1 μg of 1,25$(OH)_2$ D3 during the first 48 hr of life. We interprete this increase in plasma P in group III as the effect of 1,25-$(OH)_2$ D3 on intestinal P absorption. This confirms that there is adequate absorption of the compound in the intestine.

Several previously described characteristics of early neonatal hypocalcemia in prematures were observed in the three groups of infants in this study: rapid occurrence within the first hours (2–9), the clear-cut parathyroid response as indicated by elevated serum iPTH levels that fell to a nadir at 48 hr (7, 8) and finally the paradoxical hypercalcitoninemia that reached peak values at 24 hr and which partially to the decrease in serum Ca levels during the first 24 hr (8, 10, 20, 23).

In conclusion, early oral administration of 25-OH D3 or 1,25-$(OH)_2$ D3 to premature infants fed human milk does not significantly modify the course of early neonatal hypocalcemia as compared with infants receiving vitamin D3. This suggests that defective vitamin D metabolism is not a primary factor in the pathogenesis of early neonatal hypocalcemia. The effect observed by Chan *et al.* (5) and Fleischman *et al.* (13) with 25-OH D and 1,25-OH D are therefore pharmacological in nature.

REFERENCES AND NOTES

1. Anast, C. S., and Dirksen, H. C.: Neonatal hypocalcemia. In: A. W. Norman, K. Schaefer, J. W. Coburn, H. F. Deluca, D. Fraser, H. G. Grigoleit, D. V. Herrath. Vitamin D. Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. p. 727 (de Gruyter Publisher, Berlin, 1977).
2. Anast, C. S., and Dirksen, H. C.: Studies related to the pathogenesis of neonatal hypocalcemia. In: D. H. Copp, R. V. Talmage: Endocrinology of Calcium Metabolism. p. 12 (Excerpta Medical Foundation Publisher, Amsterdam, I.C.S. 421, 1978).
3. Barltrop, D., and Hillier, R.: Calcium and phosphorus content of transitional and mature newborn milk. Acta Paediatr. Scand., *63:* 347 (1974).
4. Bergman, L., Kjellmer, I., and Selstam, U.: Calcitonin and parathyroid hormone relation to early neonatal hypocalcemia in infants of diabetic mothers. Biol. Neonat., *24:* 151 (1974).
5. Chan, M., Tsang, R. C., Chen, I. W., de Luca, M. F., and Streichen, J. J.: The effect of 1,25$(OH)_2$ vitamin D3 supplementation in premature infants. J. Pediatr., *93:* 91 (1978).
6. David, L., and Anast, C. S.: Calcium metabolism in newborn infants: the interrelationship of parathyroid function and calcium, magnesium and phosphorus metabolism in normal, sick and hypocalcemic newborns. J. Clin. Invest., *54:* 287 (1974).
7. David, L., Salle, B. L., Chopard, J. P., and Frederich, A.: Parathyroid function in low birth weight newborns during the first 48 hours of life. In: L. Stern, B. Friis-Hansen: Symposium on Intensive Care of the Newborn. p. 107 (Masson Publishers, New York, 1976).
8. David, L., Salle, B. L., Chopard, J. P., and Grafmeyer, D.: Studies on circulating immunoreactive calcitonin in low birth weight infants during the first 48 hours of life. Helv. Paediatr. Acta *32:* 39 (1977).
9. David, L., Salle, B. L., Putet, G., Lomessy, R., and Grafmeyer, D.: Serum immunoreactive calcitonin in low birth weight infants. Description of early changes: effect of intravenous calcium infusion; relationships with early changes in serum calcium, phosphorus, magnesium, parathyroid hormone and gastrin levels. Pediatr. Res., *15:* 803 (1981).
10. Dirksen, H. C., and Anast, C. S.: Interrelationship of serum immunoreactive calcitonin (iCT) and serum calcium in newborn infants. Pediatr. Res. (Abstract), *10:* 408 (1976).
11. Dubowitz, L. M. S., Dubowitz, V., and Goldberg, C.: Clinical assessment of gestational age in the newborn infant. J. Pediatr., *77:* 1 (1970).
12. Eisman, J. A., Hamstra, A. J., Kream, B. E., and Deluca, H. F.: A sensitive, precise and convenient method for determination of 1,25-dihydroxyvitamin D in human plasma. Arch. Biochem Biophys., *176:* 235 (1976).
13. Fleischman, A. R., Rosen, J. F., and Nathenson, G.: 25-Hydroxycholecalciferol for early neonatal hypocalcemia. Occurrence in premature newborns. Am. J. Dis. Child., *132:* 973 (1978).
14. Fiske, C. H., and Subbarow, R.: The colorimetric determination of phosphorus. J. Biol. Chem., *66:* 375 (1925).
15. Gindler, E. M., and King, J. D.: Rapid colorimetric determination of calcium in biologic fluids with methylthymoblue. Am. J. Clin. Pathol., *58:* 376 (1972).
16. Glorieux, F. H., Salle, B. L., Delvin, E. E., David, L., and Senterre, J.: Serum 25-hydroxyvitamin D (25-OH) levels following vitamin D administration during the first week of life in premature infants. Pediatr. Res. (Abstract), *13:* 475 (1979).
17. Haddad, J. G., and Chyu, K. J.: Competitive protein-binding radioassay for 25-hydroxycholecalciferol. J. Clin. Endocrinol. Metab., *33:* 992 (1971).
18. Hillman, L. S., and Haddad, J. G.: Human perinatal vitamin D metabolism: 25 hydroxyvitamin D in maternal and cord blood. J. Pediatr., *84:* 742 (1974).
19. Hillman, L. S., and Haddad, J. G.: Perinatal vitamin D metabolism: serial 25 hydroxyvitamin D concentration in serum of term and premature infants. J. Pediatr., *86:* 928 (1975).
20. Hillman, L. S., Rojanasathit, S., Slatopolsky, E., and Haddad, J. G.: Serial measurements of serum calcium, magnesium, parathyroid hormone, calcitonin, and 25 hydroxyvitamin D in premature and term infants during the first week of life. Pediatr. Res., *11:* 739 (1977).
21. Prockop, P., and Udenfriend, J.: A specific method for the analysis of hydroxyproline in tissues and urine. Ann. Biochem., *1:* 228 (1960).
22. Rössli, A., and Fanconi, A.: Neonatal hypocalcemia. "Early type" in low birth weight newborns. Helv. Paediatr. Acta, *28:* 443 (1973).
23. Salle, B. L., and David, L.: Neonatal hypercalcitoninemia, in search of a physiological role. In: L. Stern, B. Friis Hansen: Intensive Care of the Newborn II. p. 217 (Masson Publisher New York, 1979).
24. Sigaard-Andersen, O., Engel, K., Jorgensen, K., and Astrup, P.: A micro-method of determination of pH, CO_2 tension, base excess and standard bicarbonate in capillary blood. Scand. J. Clin. Lab. Invest., *12:* 172 (1960).
25. Tashjian, A. H.: Immunoassay of thyrocalcitonin. I. The method and its serological specificity. Endocrinology, *84:* 140 (1969).
26. Tsang, R. C., Light, I. J., Sutherland, J. M., and Kleinman, L. T.: Possible pathogenic factors in neonatal hypocalcemia of prematurity: the role of gestation, hyperphosphatemia, hypomagnesemia, urinary calcium loss and parathormone responsiveness. J. Pediatr., *82:* 423 (1973).
27. Walton, G. M., and Garren, L. D.: An assay for adenosine 3′5′ monophosphate based on the association of the nucleotide with a partially purified binding protein. Biochemist, *42:* 23 (1970).

28. The present address of Dr. L. David is: Unité de Recherches Endocriniennes et du Métabolisme de l'Enfant, Hôpital Debrousse, 69005 Lyon, France.
29. The present address of Dr. J. Senterre is: Neonatal Department, Hôpital de Bavière, 4020 Liège, Belgium.
30. The present address of Drs. F. H. Glorieux and E. Delvin is: Genetics Unit, Shriners Hospital, Mc Gill University, Montreal, Canada.

31. The authors thank Dr. Constantine S. Anast of the Department of Pediatrics, University of Missouri, Columbia, Missouri for his invaluable gift of PTH antiserum and purified bovine PTH, Dr. Rittel and Dr. Maier (Ciba, Basel, Switzerland) for their gift of synthetic human CT and, Dr. Dietrich (Ciba, Basel) and Dr. Fisher (University of Zurich, Zurich, Switzerland) for their gift of CT antiserum. The authors wish to express their appreciation for help of the staff of the newborn nursery.

32. Requests for reprints should be addressed to: B. L. Salle, M.D., Neonatal Department, Hôpital Edouard Herriot, 69374 Lyon Cedex 2, France.

33. This study has been approved by an ad hoc research committee in the Unit Inserm U.34. Informed parental consent was obtained.
34. This research was supported by the Conseil Scientifique of University Claud Bernard-Lyon, and the Shriners of North America.
35. Received for publication December 3, 1980.
36. Accepted for publication April 24, 1981.

0031-3998/82/1601-0075$02.00/0

Printed in U. S. A.