Pediat. Res. *12:* 120-125 (1978)

Polygraphic measurements
respiratory rates, spontaneous

respiratory rates, variability
sleep states

Polygraphic Studies of Normal Infants during the First Six Months of Life. II. Respiratory Rate and Variability as a Function of State

TOKE HOPPENBROUWERS,[26] R. M. HARPER, J. E. HODGMAN, M. B. STERMAN, AND D. J. McGINTY

Newborn Division of the Los Angeles County-University of Southern California Medical Center, Department of Pediatrics-University of Southern California School of Medicine, and Sepulveda Veterans Hospital, Departments of Anatomy, Psychiatry, Psychology, and the Brain Research Institute, UCLA, Los Angeles, California, USA

Summary

This study examined spontaneous respiratory rate and variability as a function of age and sleep state in eight normal full-term infants. Each infant was admitted at 5:00 PM to the sleep laboratory for 12-hr monitoring sessions during the first week of life and at 1, 2, 3, 4, and 6 months of age. Both sleep and cardiopulmonary variables were recorded. A Beckman pCO_2 monitor sampled expired gas through a miniature cannula taped under the infant's nostrils. Peaks and troughs of breaths were measured by a computer peak sensing program. Median respiratory rates and interquartile ranges of breath intervals for each minute were determined. Each minute was coded as quiet sleep (QS), active sleep (AS), waking (AW), and indeterminate state (IN). Respiratory rates and variability were highest during the first week of life. They declined during the next 2 months and began to level out at 3 months of age (Table 3).

The state-relationship was not homogeneous at all ages. Respiratory rates and variability during wakefulness were always higher than those during sleep states. The strongest relation between respiration and sleep states was found between birth and 3 months of age. QS values were uniformly low, and those of AS and IN minutes were intermediate between AW and QS.

Speculation

Respiration rates and variability decreased linearly between birth and three months of age. The differences between the developmental course of respiratory rates and variability between 1 and 3 months reported here and cardiac rates and variability previously reported in the same infants suggest a difference in the central nervous system modulation of these systems.

Early studies of respiration in infants are characterized by a lack of systematic attention to sleep states (3, 4, 15). Monod and Pajot (14), Parmelee *et al.* (16), Roffwarg *et al.* (20), and Prechtl *et al.* (18) were among the first to establish conclusively the significant state modulation of respiratory rate and variability in infants up to 8 days of age. During the QS state, Prechtl *et al.* (18) reported breathing rates between 34 and 40/min, whereas during AS, the range increased to 42-60/min. Breath to breath variability was found to be less during QS than during AS. These studies are restricted to the newborn period.

Another variable found to influence baseline respiratory rates is the feeding or prandial cycle. Ashton and Connolly (2) found higher breathing rates during the time period immediately after feeding as compared to the remainder of the interfeeding time in all sleep states. Again, these studies were restricted to newborn infants. The lack of systematic studies in older infants has precluded assessment of the theoretical and clinical significance of early developmental respiratory patterns. A long term continuous monitoring paradigm is particularly suited for evaluation of the relative effects of state and feeding, as well as the emergence of a circadian modulation. Such normative data are essential for subsequent identification of abnormal breathing patterns.

The present report is the second in a series of papers which will describe polygraphic measurements during the first 6 months of life. It will focus upon the influence of age and sleep-waking states on respiratory rate and variability.

MATERIALS AND MONITORING PROCEDURE

Eight neurologically normal infants of gestational ages between 39 and 41 weeks and with 1-min Apgar scores of 8 or 9 participated in this study. Five were females and three were males. Birth weights ranged between 3040 and 4110 g. The infants were full-term and weights were appropriate for gestational age according to the intrauterine growth curve of Usher and McLean (23). Each infant was admitted at 5:00 PM to the sleep laboratory for 12-hr, all night monitoring sessions during the first week of life and at 1, 2, 3, 4, and 6 months of age. Informed consent was obtained from the parents. Table 1 shows the mean and SD of ages at the time of monitoring.

Monitoring was carried out in a darkened room which was adjacent to the room containing recording equipment. Room temperatures ranged between 23° and 25°. The infants were usually fed during preparation for monitoring and application of electrodes. Newborn infants were swaddled and arm restraints were applied to older infants before the initiation of recording. Sleep onset was variable among infants and ages; however, on most occasions infants fell asleep immediately. A demand feeding schedule was followed. In several instances the infant was breastfed; this did not interrupt the monitoring. The infants were observed continuously during recording with the use of a low illumination television camera and monitor. Behaviors such as closing or opening of eyes, startles, crying and vocalization, and nursing interventions were charted on the polygraphic paper.

PHYSIOLOGIC RECORDING METHOD

Both sleep and cardiopulmonary variables were monitored. The former included two EEG derivations, a chin electromyogram, eye movements, and gross somatic activity (9). Thoracic

or abdominal excursions were monitored by impedance pneumography (Gould, Inc., Instrument Systems Division, Los Angeles, CA). Two disposable electrodes were placed bilaterally on either side of the chest or abdomen, depending on which area showed the largest respiratory excursion during normal breathing. In addition, a Beckman pCO_2 monitor sampled expired gas through a miniature cannula taped under the infant's nostrils (Beckman Instruments, Palo Alto, CA). In order to adjust for the timelag inherent to this instrument, air passage was simultaneously detected with a thermistor placed into one arm of the cannula.

Data were recorded on a 16-channel Grass model 76 polygraph (Grass Instruments Co., Quincy, MA) and simultaneously stored on a 14-channel Honeywell analog tape recorder (Honeywell, Test Instruments Co., Quincy, MA), together with an IRIG E time code (Systron Donner Corp., Concord, CA) for future computer analysis.

ANALYTIC METHODS

Data on the analog tapes were processed by a PDP-12 computer (Digital Equipment Corp., Maynard, MA). The respiratory signal derived from the three respiratory measures was digitized at 8 samples/sec and stored on industry-compatible magnetic tape, together with digitized values of other physiologic measures. Although three estimates of respiration were obtained, the pCO_2 signal proved to be the most useful for this analysis, since movements of the infant did not greatly affect the signal quality. Peaks and troughs of breaths were measured by a peak sensing program developed by Mason *et al.* (10). Breathing intervals were then determined as the time between peaks. As expired CO_2 rather than tidal volume was being measured, the peak as identified by the CO_2 sensor was not necessarily a fixed point in the breathing cycle. The potential error introduced by this methodology is negligible compared to the magnitude of the respiratory variability across 1 min (Fig. 1). Rates were determined for the entire 12-hr period. The interquartile range of these same intervals was chosen as a measure of respiratory variability, expressed in breaths per min. Minute-by-minute values for median respiratory rate and varability over the entire 12 hr (720 data values) were plotted on an incremental plotter (Houston Instruments, Bellaire, TX).

Each minute of the record was coded by trained personnel into QS, AS, AW, or IN. Although sleep states can be easily identified both behaviorally and physiologically in adults, infant recordings pose greater problems in sleep state definition. Sleep spindles, reliable indicators of quiet sleep in adults, are an emerging phenomenon in infancy (12, 13, 21). Muscle tone does not reliably discriminate between sleep states in the young infant. Respiratory patterns, especially regularity of breathing, proved to be useful for sleep state definition in the young infant, and were therefore employed in this study. State criteria and decision-making rules are described elsewhere (9), and were chosen from criteria recommended by other investigators (1). The state codes were stored in a file on digital tape for correlation with respiration rate and variability data. Subsequently, group means of rate and variability for each state were calculated.

When the infant cried for an extended period, the pCO_2

Table 1. *Mean and SD of postnatal ages in eight normal full-term infants*

Monitoring interval	Mean age, days	SD
1 week	4.8	2.0
1 mo	32.4	4.3
2 mo	62.4	3.8
3 mo	94.1	8.2
4 mo	125.2	10.5
6 mo	177.0	13.1

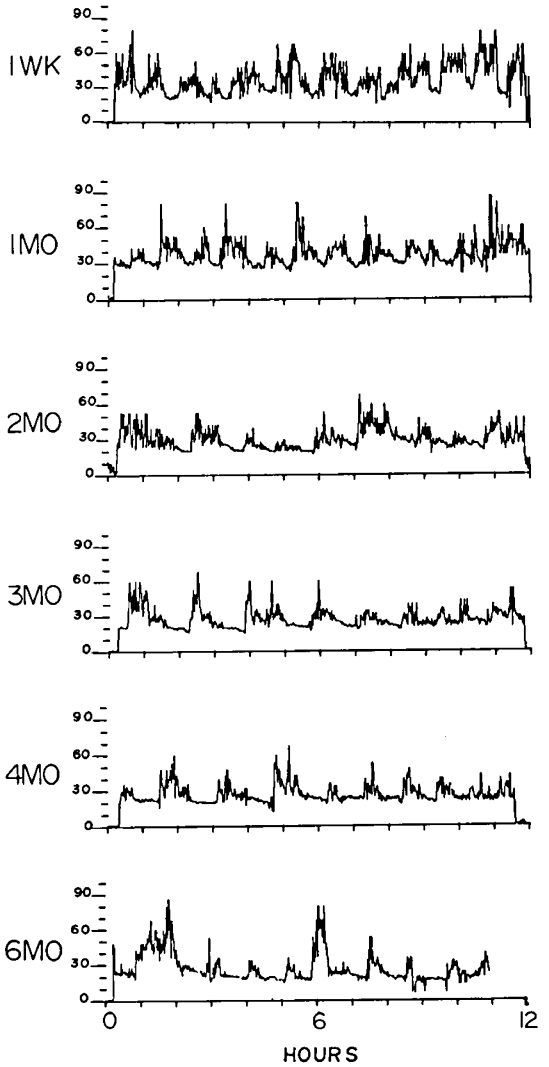

Fig. 2. These graphs represent the minute-by-minute respiratory rates in one infant across the entire agespan under investigation. The ordinate represents median breaths per min and the abscissa, hours. Note the decrease in respiratory rates beteen 1 week and 6 months.

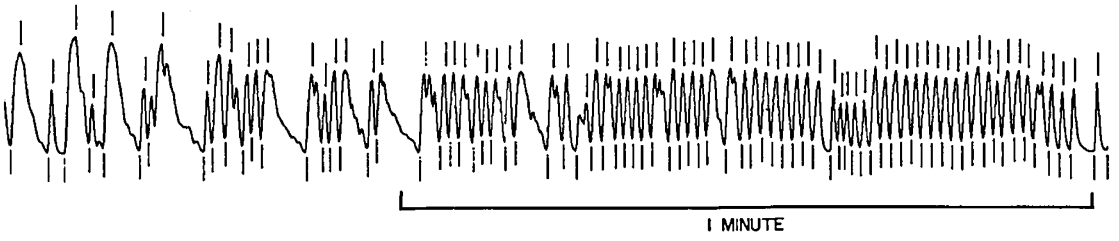

Fig. 1. Performance of computer program which identified peaks and troughs of respirations (12). This sample was obtained during wakefulness when respiration rate is typically high and the pattern irregular.

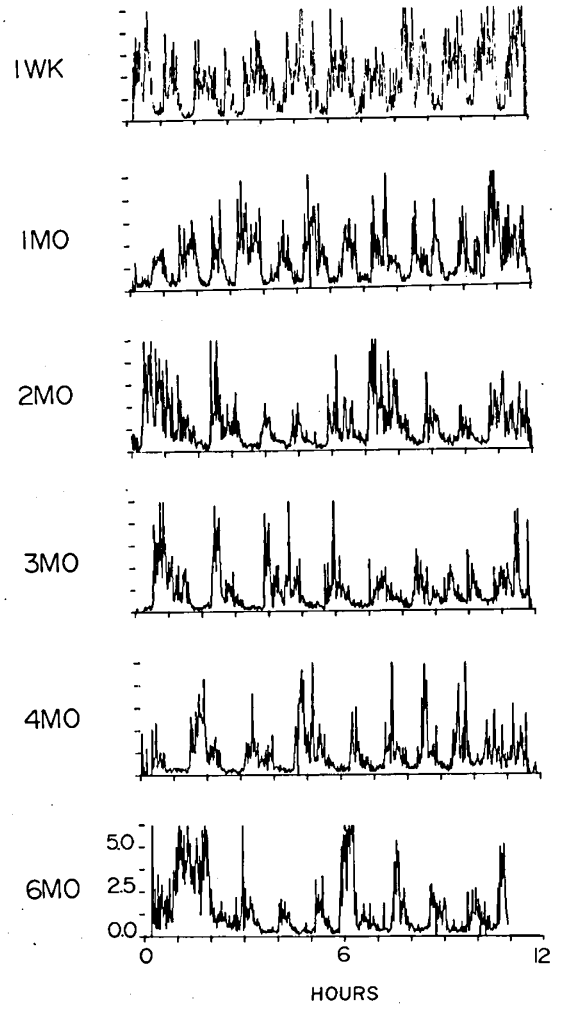

Fig. 3. Minute-by-minute plots of respiratory variability as measured by the interquartile range. Data are from the same infant as in Figure 2. The ordinate represents breaths per min. The variability is highest in the newborn period. The periodic fluctuations in respiratory variability are more pronounced than those in respiratory rate.

respiratory tracing occasionally disappeared entirely, indicating a complete shift to mouth breathing. The resulting long respiratory pause would give erroneous results. To deal with this problem, long episodes of crying were deleted from the analysis. When crying episodes were less than 3 successive minutes in duration and the signal disappeared, the mean of the preceding and the following minute was used as interpolated value. The respiratory signal was almost always preserved (although with lower amplitude) throughout short cries and vocalization and could thus be calculated. The use of median rather than mean values provided some degree of protection against aberrant data. In all cases, the polygraphic records and, in particular, the chart notations and the impedance respiratory signal, provided final reference for artifact evaluation. Except for those minutes of artifactual data removed (ranging between 0% and 8%), respiratory intervals over the entire 12-hr record were used to derive results.

Developmental trends and the effects of state on respiratory rate and variability were assessed with an analysis of variance (6) and a multiple regression program. The Duncan multiple range test was used to evaluate selective mean values at a 5% level of significance (7).

RESULTS

The respiratory rate in infants at all ages studied exhibited periodic fluctuations. Figure 2 shows median respiratory rates across six 12-hr monitoring sessions for one representative infant. Peaks in respiratory rate occurred approximately every hour in the newborn and 1-month recording. At subsequent ages intervals between peaks increased. Minute-by-minute respiratory variability, as measured by the interquartile range, is shown in Figure 3. Periodic modulation throughout the 12-hr records was again pronounced. Respiratory rate and variability decreased across the agespan studied. Respiratory rate and variability were greatest when the infant was awake, lowest during QS, and intermediate during AS (Fig. 4).

When group means for each age and state were compared, a developmental trend emerged, as presented in Figure 5. The results of the analysis of variance are outlined in Table 2. Comparisons of individual means revealed that respiratory rates were highest during the first week of life. A sharp decline occurred during the next 2 months; rates at 3, 4, and 6 months were similar. Variability in breathing also showed a decline and values again leveled out at 3 months of age.

The respiratory parameters under investigation showed state-related characteristics, but they were not homogeneous at all ages. Respiratory rates during the awake state were always significantly higher than the rates determined from sleep states, except during the first week of life. At this early point in development, the rates for AS and AW were similar. During sleep respiratory rates in QS represented the lowest values recorded for the first 2 months of life ($P < 0.05$). Thereafter, rates in QS differed significantly from AS, but not from IN episodes. Between 3 and 6 months of age, state-related differences were minimal. At 6 months of age, values began to diverge again. The rates during IN epochs most resembled AS rates at all ages. At 3 and 4 months, however, the IN rates could not be reliably differentiated from the respiratory rate during QS epochs.

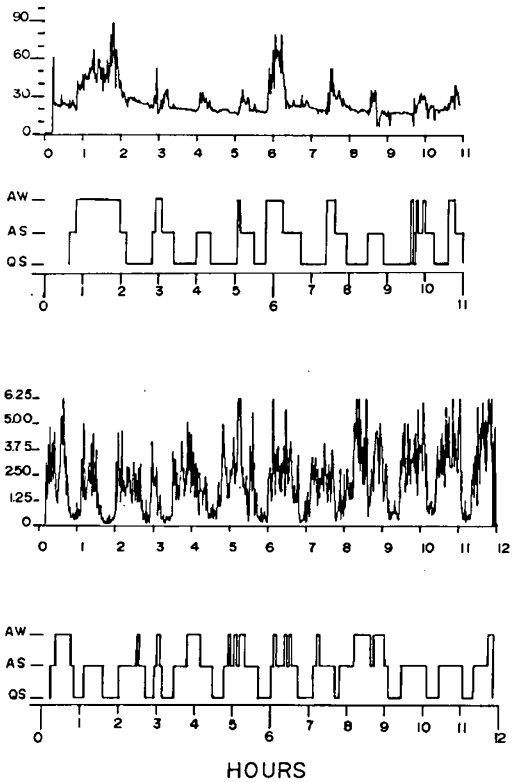

Fig. 4. The plot of state codes assigned to each minute was compared with the minute-by-minute respiratory rate (*top*) and variability (*bottom*) graphs. The highest respiratory rates were observed during periods of wakefulness, followed by rates during periods of active sleep. Similarly, variability values were maximal during AW, lowest during QS, and intermediate during AS.

Breathing was most variable during the waking state across the entire agespan studied. Variability during QS was significantly lower than during all other states, except at 3 months, when variability during QS and IN epochs was similar. Respiratory variability during IN epochs in the newborn period was intermediate between QS and AS values and significantly different from either of these states ($P < 0.05$).

Individual differences in respiratory rate were large, especially during the first 2 months. At 3 months values became more homogeneous during sleep, as can be seen from the SD in Table 3. A correlation between sex or birthweight could not be established.

The similarity between the developmental curves of rates and variability led us to examine the effect of rate on variability. In order to assess whether or not developmental changes in variability were dependent on concurrent changes in rate, variability was examined at 25 and 30 breaths/min at each age. This analysis demonstrated that variability decreased independent of respiratory rate between birth and three months of age in AS; however, in QS variability remained the same once rate was controlled.

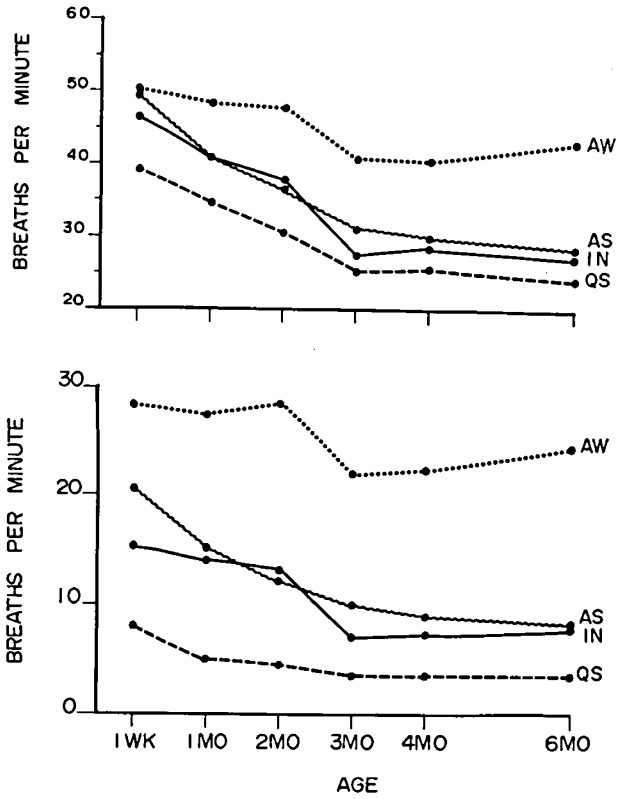

Fig. 5. Group means of the median respiratory rates are plotted as a function of age and state (*top*). Note the decline in breaths per min with increasing age across all states. The group means of respiratory variability (*bottom*) were highest during wakefulness and lowest during QS. AS and IN were characterized by an intermediate variability in respiration.

DISCUSSION

From birth to 3 months of age respiratory rates and variability decreased in all states. Values were clearly state-related with the highest rates and largest variability observed during wakefulness, and the lowest during QS. From 3-6 months of age the infants were surprisingly stable. First, no developmental changes in respiratory rate and variability were observed. Second, the infants began to resemble a homogeneous group during sleep, with the large intrasubject differences restricted to wakefulness.

During the first week of life we found a mean respiratory rate during QS and AS of 38.2 and 50.5 breaths per min, respectively. These rates are comparable to those reported by Prechtl *et al.* (19), Theorell *et al.* (22), and Ashton and Connolly (2). Values for wakefulness can also be compared to those reported in earlier studies. Waking respiratory rates during the first week of life obtained by Murphy and Thorpe (15) ranged between 42 and 76 breaths per min, which agrees well with the range of 40–68 in our study. Deming and Washburn (4) found a wider range of waking respiratory rates, from 46–108 breaths per min, with a mean of 59 in a group of infants of variable age studied from 1 day to 13 weeks. We found mean rates of 50, 48.1, and 47.6 during the first 2 months with a decrease to 40.6 breaths per min between 2 and 3 months. Paul *et al.* (17) focused upon QS rates alone. As infants were monitored repeatedly up to 3 months of age, this study is the most comparable to ours. These authors stressed differences between respiratory rates at the beginning and end of a QS episode. They reported stable average rates of 28 breaths per min at the termination of QS episodes from 6 weeks of age on. Average QS onset values increased between 6 weeks and 5 months of age and ranged between 32 and 37 breaths per min. The results obtained in our study lie between these values, a difference which can probably be explained by the disparity in QS episodes sampled in the two studies. Paul *et al.* (17) monitored rates during three cycles after evening feeding, whereas the rates reported here were average values over 12 hr for as many as seven or eight sustained QS episodes. During the ages examined by us a modulation of QS respiratory rates emerged with lowest rates between 10:00 PM and 3:00 AM; these minima were included in the averages reported here.

Table 2. *Variances for mean respiratory rate and variability*

Source	SS	df	MS	*F*	*P*≤
Mean respiratory rate					
Main effect A: sleep state	5,807.50	3	1,935.83	68.67	0.001
A × subjects within groups	592.02	21	28.19		
Main effect B: age	6,973.42	5	1,394.68	28.13	0.001
B × subjects within groups	1,735.09	35	49.57		
Interaction A × B	631.58	15	42.11	4.99	0.001
A × B × subjects within groups	886.29	105	8.44		
Mean respiratory variability					
Main effect A: sleep state	10,944.40	3	3648.13	206.34	0.001
A × subjects within groups	371.28	21	17.68		
Main effect B: age	1,496.03	5	299.21	26.31	0.001
B × subjects within groups	398.05	35	11.37		
Interaction A × B	354.28	15	23.62	2.27	0.008
A × B × subjects within groups	1,094.03	105	10.42		

[1] SS: sum of squares; df: degrees of freedom; MS: mean square.

Table 3. *Median respiratory rate during sleep and waking states*

Subjects	Sex	Age 1 wk	1 mo	2 mo	3 mo	4 mo	6 mo
AS							
1	F	43.4	36.7	32.8	30.0	29.6	25.8
2	F	41.3	40.0	29.7	29.7	27.3	25.3
3	M	47.5	42.9	34.4	27.4	29.1	31.3
4	F	53.0	42.9	42.8	32.4	28.7	30.9
5	M	49.4	41.9	46.2	33.7	28.8	26.6
6	F	45.4	33.2	32.3	26.4	26.8	25.6
7	F	59.2	41.2	32.2	30.6	31.0	24.8
8	M	64.8	55.1	39.8	36.0	36.7	34.2
Mean		50.5	41.7	36.3	30.8	29.8	28.1
SD		8.1	6.4	5.9	3.2	3.1	3.5
QS							
1	F	30.0	31.0	26.7	23.7	25.1	22.3
2	F	26.6	30.4	24.0	21.4	22.2	19.7
3	M	31.0	35.6	28.0	24.8	26.0	23.1
4	F	45.7	36.9	39.2	27.1	25.5	26.6
5	M	42.4	33.4	35.8	28.8	24.0	21.5
6	F	37.8	31.0	27.7	21.3	23.5	20.2
7	F	39.4	34.3	26.6	24.9	25.7	22.4
8	M	52.7	42.0	34.2	28.8	31.6	31.4
Mean		38.2	34.3	30.3	25.1	25.5	24.0
SD		8.8	3.9	5.4	3.0	2.8	4.2
IN							
1	F	40.6	38.9	28.8	27.3	29.7	24.2
2	F	35.7	36.7	34.0	24.2	25.4	23.6
3	M	41.3	45.7	38.4	26.8	29.0	32.6
4	F	48.2	42.7	52.2	29.1	29.1	35.1
5	M	44.4	38.3	43.5	28.6	26.7	22.5
6	F	42.4	32.0	32.3	23.0	25.5	23.2
7	F	50.1	44.4	27.8	28.2	28.0	25.1
8	M	69.7	53.3	41.3	29.4	33.0	36.0
Mean		46.6	41.5	37.3	27.1	28.3	27.8
SD		10.4	6.5	8.3	2.3	2.5	5.7
AW							
1	F	42.7	43.1	47.9	40.0	39.0	43.1
2	F	45.5	43.7	42.6	38.5	39.0	46.0
3	M	46.9	54.0	51.9	40.0	45.6	42.9
4	F	59.2	55.7	54.9	41.6	35.9	43.3
5	M	40.4	47.2	53.8	43.1	33.6	32.8
6	F	44.3	43.2	40.4	32.9	31.3	34.5
7	F	52.6	42.8	35.9	32.0	36.0	37.3
8	M	68.1	55.2	53.7	56.6	60.5	63.3
Mean		50.0	48.1	47.6	40.6	40.1	42.9
SD		9.5	5.9	7.2	7.6	9.3	9.5

An increase in heart rate after feeding has been reported in the 1-week-old and 1-month-old infants in this series (11). Respiratory rates were also higher after feeding and, since the time between feedings lengthens as the infant grows older, one would expect some decrease in respiratory rate. The maturational changes in respiratory rate described here include the effects of normal feeding patterns during the night.

Respiratory rates and variability were strikingly similar, both in terms of the course of development and sleep state correlation (Figs. 5 and 6). Although respiratory variability was used as a criterion for state, this criterion was identical for all age groups examined; this obvious circularity with respect to state definition should not obscure the significance of changes observed over age. Variability was found to decrease independently of rate between 1 week and 3 months, but only in AS. The infant not only spent a larger percentage of time in AS during the first weeks of life, but the quality of AS was different.

The underlying mechanism and the relationship between state and respiration are of clinical interest. Both state and respiratory parameters seem to be affected by similar pathologic conditions in infancy. Radvanji *et al.* (19) studied premature infants wtih and without assisted ventilation. Assisted ventilation led to an increase in AS, whereas abnormal biochemical conditions such as base deficit and acidosis led to an increase in QS. Dittrichova *et al.* (5) established that respiratory rates were still faster at 6 months in premature infants who had RDS and in small for date infants as compared to full-term and healthy premature infants.

The maturation of respiration described here followed a different course than that of cardiac measurements described earlier in the same infants (9). Although a quadratic function provided the best fit for the developmental course of heart rate and variability, a linear function best described the maturation of respiration. These results agree with the report of Evsywkova (8), who in a study of full-term infants found an initial increase in heart rate between birth and 10 days of age, and a steady decrease in respiratory rates. The difference between cardiac and respiratory ontogenesis raises questions about the functional relationship between these vegetative systems during development. A transient dissociation between 1 and 3 months of age in the development of central nervous system control mechanisms for these two functions is one hypothesis to explain this observation. Another could invoke differential rate of maturation of vagal and sympathetic nerves.

Two considerations resulting from these findings deserve emphasis or further study. First, the sleep, waking state of the infant must be considered in order to accurately evaluate respiratory function. Secondly, on the basis of respiratory variability, the first weeks of life can be considered unlike any subsequent age. This suggests that there are at least three relatively discreet stages of development: a newborn period, early infancy (1-3 months), and later infancy.

CONCLUSION

Spontaneous respiratory rate and variability were determined as a function of age and sleep state in eight normal full-term infants, from birth to 6 months of age. Respiratory rates were highest during the first week of life, decreased linearly between birth and 3 months of age, and stabilized thereafter. Respiratory variability followed a similar pattern. The strongest correlations between respiration and sleep states were found between birth and 3 months of age. AW was characterized by the highest values for both rate and variability and QS by the lowest values. The values for IN most resembled those of AS at all ages except at 3 and 4 months, when rates for IN epochs resembled QS states. Variability values for the IN epochs were different from those of both AS and QS in the 1-week recordings. AS was accompanied by a relatively greater respiratory variability in the first week of life than at older ages. Since the regulation of respiratory activity is greatly modified by sleep and waking behavior, states must be considered in the measurement of these parameters.

REFERENCES AND NOTES

1. Anders, T., Emde, R., and Parmelee, A.: A Manual of Standardized Terminology, Techniques and Criteria for Scoring of States of Sleep and Wakefulness in Newborn Infants. (UCLA Brain Information Service/BRI Publications Office, Los Angeles, 1971).
2. Ashton, R., and Connolly, K.: The relation of respiration rate and heart rate to sleep states in the human newborn. Develop. Med. Child. Neurol., *13:* 180 (1971).
3. Boutourline-Young, H. J., and Smith, C. A.: Respiration of full term and premature infants. Amer. J. Dis. Child., *80:* 753 (1950).

4. Deming, J., and Washburn, A. H.: Respiration in infancy. I. A method of studying rates, volume and character of respiration with preliminary report of results. Amer. J. Dis. Child., *49:* 108 (1935).
5. Dittrichova, J., and Paul, K.: Respiratory rate during quiet sleep in high risk infants during the first six months of life. In: P. Levin and W. P. Koella: Sleep 1974: Second European Congress on Sleep Research, Rome, 1974, p. 417 (Karger, Basel, 1975).
6. Dixon, W. J.: Biomedical Computer Programs (University of California Press, Berkeley, 1975).
7. Edwards, A. L.: Experimental design in psychological research, 3rd Ed. p. 441 (Holt, Rinehart and Winston, 1968).
8. Evsywkova, I.: Heart rate and respiration in newborn infants during different phases of sleep. Z. Evol. Biokhim. Fiziol., *10:* 267 (1974).
9. Harper, R. M., Hoppenbrouwers, T., Sterman, M. B., McGinty, D. J., and Hodgman, J. E.: Polygraphic studies of normal infants during the first six months of life. I. Heart rate and variability as a function of state. Pediat. Res., *10:* 945 (1976).
10. Mason, J., Harper, R. M., and Pacheco, R.: Analysis of respiratory data during sleep and waking. Proc. Dig. Equip. Comput. Users Soc., p. 567 (1974).
11. Harper, R. M., Hoppenbrouwers, T., Bannett, D., Hodgman, J. E., Sterman, M. B., and McGinty, D. J.: Effects of feeding on state and cardiac regulation in the infant. Develop. Psychobiol. (In press).
12. Lenard, H. G.: The development of sleep spindles in the EEG during the first two years of life. Neuropädiatrie, *1:* 264 (1970).
13. Metcalf, D. R.: Sleep spindle ontogenesis. Neuropädiat., *1:* 428 (1970).
14. Monod, N., and Pajot, N.: Le sommeil du nouveau-né et du prematuré I. Analyse des études polygraphiques. Biol. Neonate, *8:* 281 (1965).
15. Murphy, D. P., and Thorpe, E. S.: Breathing measurements on normal newborn infants. J. Clin. Invest., *10:* 545 (1931).
16. Parmelee, A. H., Wenner, W. H., Akiyama, Y., Schultz, M., and Stern, E.: Sleep states in premature infants. Develop. Med. Child. Neurol., *9:* 70 (1967).
17. Paul, K., Dittrichova, J., and Pavlikova, E.: The course of quiet sleep in infants. Biol. Neonate, *23:* 78 (1973).
18. Prechtl, H. F. R., Weinmann, H., and Akiyama, Y.: Organization of physiological parameters in normal and neurologically abnormal infants. Neuropädiatrie, *1:* 101 (1969).
19. Radvanji, M. F., Monod, N. and Dreyfus-Brisac, C.: Sleep cycle organization in newborn babies with respiratory distress syndrome. In: P. Levin and W. P. Koella: Sleep 1974. Second European Congress on Sleep Research, Rome, 1974, p. 428 (Karger, Basel, 1975).
20. Roffwarg, H. P., Muzio, J. N., and Dement, W. C.: Ontogenetic development of the human sleep-dream cycle. Science, *152:* 604 (1966).
21. Sterman, M. B., Harper, R. M., Havens, B., Hoppenbrouwers, T., McGinty, D. J., and Hodgman, J. E.: Quantitative analysis of central cortical EEG activity during quiet sleep in infants from birth to six months of age. Electroencephal. Clin. Neurophysiol., *43:* 371 (1977).
22. Theorell, K., Prechtl, H. F. R., and Vos, J. E.: A polygraphic study of normal and abnormal newborn infants. Neuropädiatrie, *5:* 279 (1974).
23. Usher, R., and McLean, F.: Intrauterine growth of live-born Caucasian infants at sea level: Standards obtained from measurements in seven dimensions of infants born between 25 and 44 weeks of gestation. J. Pediat., *74:* 901 (1969).
24. The authors acknowledge the assistance of the Biomedical Engineering Departments and the Computing Center at the VA Hospital in Sepulveda. We thank Dr. E. Gocka and Mr. Leeds for their statistical help. J. R. Mason, D. Bannett, and D. Hockin were in charge of computer analysis. Ms. Havens, Hofmann, and Geidel shared responsibility for the monitoring laboratory and together with other specially trained nurses collected the data.
25. This research was supported by the National Institute of Child Health and Human Development Contracts NO1-HD-2-2777 and HD 4-2810.
26. Requests for reprints should be addressed to: T. Hoppenbrouwers, Ph.D., Director Sudden Infant Death Syndrome (SIDS) Research Project, Room 4L40B, Women's Hospital, LAC/USC Medical Center, 1240 Mission Road, Los Angeles, CA 90033 (USA). [Tele: (213) 226-3266; (213) 894-8271, ext. 2403.]
27. Received for publication September 14, 1976.
28. Accepted for publication May 25, 1977.

0031-3998/78/1202-0120$02.00/0
Copyright © 1978 International Pediatric Research Foundation, Inc.

Printed in U.S.A.

Pediat. Res. *12:* 125–133 (1978)

Cystathionase
cystathionine
cystathioninuria
genetic heterogeneity
long term lymphoid lines
pyridoxalphosphate
vitamin-responsive amino aciduria

Cystathionase Deficiency: Evidence for Genetic Heterogeneity in Primary Cystathioninuria

THERESA A. PASCAL,[49] GERALD E. GAULL, NICHOLAS G. BERATIS, BRUCE M. GILLAM, AND HARRIS H. TALLAN

Department of Human Development and Genetics, New York State Institute for Basic Research in Mental Retardation, Staten Island, and Division of Medical Genetics, Department of Pediatrics, Mount Sinai School of Medicine of the City University of New York, New York, USA

Summary

Optimal conditions are described for measurement of cystathionase activity in long term lymphoid cell lines. In 21 control lines established from normal subjects, cystathionase (EC 4.4.1.1) specific activity was 25.8 ± 1.7 (mean ± SE) nmole cysteine/hr/mg protein. Extracts of three lymphoid lines (NB-36, NB-95, and NB-77) established from three vitamin B_6-responsive patients with primary cystathioninuria had activity of 3.6–7.3; from a B_6-unresponsive patient (NB-68) had no detectable activity; from five obligate heterozygotes for cystathioninuria had activity of 11.2–18.9.

Two, or possibly three, different modifications of the cystathionase molecule could be demonstrated in cultured cells from the patients with primary cystathioninuria, based on the effects of added pyridoxal phosphate (PLP) in the enzyme assay, on the extent of reaction with rabbit antihuman hepatic cystathionase, and on the ability to compete with normal lymphoid cell line enzyme extract for the antibody combining sites. When PLP is not added to the assay system, the normal enzyme extract still had 89% of its activity in 1.0 mM PLP; on agar double diffusion analysis it gave a band of identity with normal human hepatic cystathionase; the precipitin band had cystathionase activity, but inhibition by antibody could be shown in solution. Lymphoid line extract from the B_6-unresponsive patient had no detectable activity in the absence or presence of PLP, did not form a precipitin band, and did not compete with normal enzyme extract for the antibody combining sites. Thus, synthesis of apocystathionase is absent or significantly reduced; alternatively, the protein produced has lost its antigenic determinants