structured polynucleotides with one another or with other polymers. For instance, conventional spectrophotometric methods (mixing curves, melting temperatures), as expected, fail to reveal any interaction of the highly ordered poly(G) with poly(A)·poly(U); yet poly(G) brings about the same dramatic decreases in interferon titer as does poly(U) or poly(I). One possible explanation, the poly(A) • poly(U) • poly(G) triplex, would considerably expand the scope and importance of triple-stranded structures in biological systems.

Thus, interferon induction studies suggest that triple-stranded complexes among complementary homopolyribonucleotides are readily formed both in solution and at the cellular level. Further investigation will be required to determine whether similar RNA triplexes (or their DNA equivalents) may possess a biological function (*17*), for example, in the tertiary structure of RNA or control of gene expression (*18*).

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19. To enhance the yield of interferon, we "superinduced" the cells with cycloheximide and actinomycin D. Immediately after the cells' exposure to the polymers, the cells were treated with cycloheximide (2 μg/ml in MEM containing 3 percent calf serum, 2 ml per petri dish) for 3 hours at 37°C, washed three times with MEM, treated with actinomycin D (3 μg/ml, in MEM containing 3 percent calf serum, 2 ml in each petri dish) for ½ hour at 37°C, washed again (three times) with MEM and further incubated with MEM containing 3 percent calf serum (4 ml per petri dish) for 20 hours at 37°C. The supernatants of these cell cultures were then withdrawn and titrated for interferon (*4*).
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Hypoxemia and the Sudden Infant Death Syndrome

Abstract. *Infants with known chronic hypoxemia before death retained a large proportion of the brown fat cells that are normally replaced by white fat cells after birth. Many of these hypoxemic infants also had an abnormal retention of extramedullary hematopoiesis. These same abnormalities were found in many victims of the sudden infant death syndrome.*

Prolonged apneic periods during sleep, accompanied by cyanosis, have been described in two sudden infant death syndrome (SIDS) victims prior to death (*1*). In several adult disorders such episodes of sleep apnea are associated with chronic alveolar hypoventilation (*2*). Recently it was found that the small pulmonary arteries of most SIDS victims have more muscle than do those of nonhypoxic controls (*3*). This vascular abnormality is a characteristic consequence of chronic alveolar hypoventilation (*4*). Since such hypoventilation induces arterial hypoxemia, it is of importance to determine whether SIDS victims have markers of chronic hypoxemia. Brown fat retention appears to be one possible marker and persistent extramedullary hematopoiesis is another.

Periadrenal brown fat is normally replaced by white fat during the first year of postnatal life. It has recently been reported that brown fat reappears in adults who are chronically hypoxemic (*5*). The purpose of our study was to determine whether brown fat and extramedullary hematopoiesis are good markers for chronic hypoxemia in early infancy and if these markers are present in SIDS victims.

The patients included 65 infants, 1 month to 1 year in age, who were categorized as having died of SIDS when the death was sudden and unexplained by any clinical or postmortem findings. Also included were 26 infants who were placed in the SIDS category with pulmonary inflammation when the infants had bronchopneumonia, tracheobronchitis, laryngitis, or interstitial

Table 1. Percent of periadrenal fat cells that are brown. The numbers of cases are shown in parentheses; all values are ± 1 standard deviation of the mean.

Subjects	Brown fat (%) at age		
	1 to 2 months	2.1 to 5 months	5.1 to 12 months
Nonhypoxic controls	91 ± 10 (17)	60 ± 24 (17)	41 ± 25 (14)
SIDS			
No pulmonary inflammation	93 ± 7 (26)	90 ± 18 (31)*	78 ± 22 (8)*
Pulmonary inflammation	89 ± 17 (8)	84 ± 6 (12)*	41 ± 26 (6)
Hypoxemic controls			
Cyanotic congenital heart disease	88 ± 4 (6)	80 ± 11 (6)*	74 ± 5 (6)*
Wilson-Mikity syndrome	89 ± 11 (6)	91 ± 9 (4)†	
Werdnig-Hoffmann disease		79 (2)	
Patients with central nervous system lesions		82 ± 5 (3)†	88 ± 9 (4)*

* $P < .02$ by comparison with nonhypoxic controls of same age. † $P < .05$ by comparison with nonhypoxic controls of same age.

pneumonia of a degree too mild to explain death (*3*). Three types of chronically hypoxemic controls were examined: (i) 18 infants with cyanotic types of congenital cardiac anomalies; (ii) ten infants with Wilson-Mikity syndrome, a severe pulmonary disorder that led to chronic hypoxemia in all ten cases; and (iii) two infants with Werdnig-Hoffmann disease in whom muscle weakness led to severe hypoventilation and chronic hypoxemia. An additional six infants had severe central nervous system abnormalities that led to death; four had diffuse gliosis, one had hydrocephalus, and one had porencephaly. No information about the gas levels in their blood is available. Also included in the study were 48 nonhypoxic controls. They were victims of accidents, homicides, or acute infections. None lived longer than 24 hours after the onset of illness or accident.

Periadrenal fat cells with a clearly defined cytoplasmic reticular infrastructure were counted as brown cells. The proportion of fat cells that were brown was determined, in each case, by the point counting method of Chalkley (*6*). Measurements were made in duplicate by two examiners without either knowing an infant's age or diagnosis. Nonadipose structures, such as blood vessels and connective tissue, were excluded from the final calculations so that brown cells were represented as a percentage of the total volume of fat cells present. Extramedullary hematopoiesis was diagnosed when nests of hematopoietic cells, including normoblasts, were identified between the cords of hepatic parenchymal cells. The amount of such hematopoiesis was graded by counting the numbers of such nests per microscopic field. Results of the brown fat and hematopoietic analyses were compared with measurements of muscle mass in small pulmonary arteries. The methods used to measure the arteries have been described (*3*). Student's *t*-test, chi-square, and simple linear regression were used to analyze data in the study.

In the infants in all groups, most of the periadrenal fat cells were brown in type during the first 2 months of postnatal life (Table 1). About 40 percent of these fat cells became white in type between 2 and 5 months after the infants in the nonhypoxic control group were born (Table 1). By contrast, there was no significant decrease in the proportion of brown fat cells in the SIDS victims and cyanotic controls during this period. A further but slower shift from brown to white cells took place between ages 5 to 12 months in the nonhypoxic controls. As in the earlier period, most of the fat cells remained brown in type in the SIDS patients without pulmonary inflammation and in the cyanotic congenital heart victims. Infants with known severe central nervous system abnormalities also had an increased retention of brown cells (Table 1). In the small pulmonary arteries the mean mass of medial smooth muscle was 38 percent greater than that of nonhypoxic controls ($P < .05$). The two infants known to have had chronic alveolar hypoventilation before death (Werdnig-Hoffmann disease) had an abnormal retention of brown fat (Table 1). The muscle mass about their small pulmonary arteries was 101 percent greater than that found in the nonhypoxic controls. A significantly greater proportion of the SIDS victims had hepatic extramedullary hematopoiesis than did the nonhypoxic controls (Table 2).

Table 2. Percentage of patients in a category with extramedullary hematopoiesis in the liver.

Subjects	Percent
Nonhypoxic controls	0
SIDS	
No pulmonary inflammation	16*
Pulmonary inflammation	17*
Hypoxemic controls	
Cyanotic congenital heart disease	12
Werdnig-Hoffmann disease	50
Patients with central nervous system lesions	38*

*$P < .05$ by comparison with nonhypoxic controls.

There was a correlation between increased brown fat retention and an increased amount of smooth muscle about the small pulmonary arteries in the SIDS victims who had no pulmonary inflammation ($r = .45$). A similar correlation was found between arterial muscle values and the hepatic extramedullary hematopoiesis when the latter was graded from 0 to 3+ in individual cases ($r = .65$). There was no increase in brown fat retention in any of the clinical or age categories during the colder months of the year.

Part of newborn infants' normal thermogenic response to cold exposure derives from brown fat. In normal infants this brown fat is gradually replaced by white fat during the first year of life. Also, as shown here, chronic hypoxemia retards brown fat replacement during this period. Brown cells were also abnormally retained in many SIDS victims, an indication that some of them may have experienced chronic hypoxemia before death (*1, 3*). The abnormal retention of small foci of extramedullary hematopoiesis in some SIDS victims is also an indicator of previous long-term hypoxemia. Chronic alveolar hypoventilation is the most likely cause of hypoxemia in such cases because increases in the retention of brown fat and extramedullary hematopoiesis were accompanied by increases in smooth muscle about the small pulmonary arteries. An increased mass of muscle in these arteries is a sensitive indicator of chronic alveolar hypoxia in the absence of cardiac anomalies that raise pulmonary blood flow or pressure (*4*). None of the SIDS victims had such cardiac anomalies.

Brown fat was abnormally retained and pulmonary arterial muscle increased in six infants who died with severe central nervous system abnormalities. This finding raises the possibility that central neural mechanisms of respiratory control might be immature or abnormal in some SIDS victims. These control mechanisms may be affected by respiratory infections since such infections prolong episodes of sleep apnea and cyanosis in the early months of life (*1, 7*). This effect of infections on respiration may explain why SIDS victims who have respiratory infections have less brown fat and pulmonary arterial muscle than do SIDS victims who have no evidences of infection. Chronic abnormalities in respiratory control may be less common in SIDS victims whose deaths were accompanied by respiratory infections.

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