13. A. Heller and R. Y. Moore, *Adv. Pharmacol.* **6A**, 191 (1968).
14. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
15. J. C. Schwartz, C. Rose, H. Caillens, *J. Pharmacol. Exp. Ther.* **184**, 766 (1973).
16. M. Medina and P. A. Shore, *Biochem. Pharmacol.* **15**, 1627 (1966).
17. M. J. Kuhar, R. H. Roth, G. K. Aghajanian, *Brain Res.* **35**, 167 (1971).
18. M. Garbarg, M. S. Krishnamoorthy, J. Feger, J. C. Schwartz, *ibid.* **50**, 361 (1973).
19. When a more detailed dissection of the telencephalon was performed, significant reductions in enzyme activity were observed in all tested regions including septum, olfactory bulb, and cortex divided in five separate areas. It seems unlikely that the decline in L-histidine decarboxylase was secondary to degeneration of noradrenergic or serotoninergic neurons since specific chemical destruction of these two systems by intraventricular administration of 6-hydroxydopamine and 5,6-dihydroxytryptamine, respectively, failed to induce such effect (J. C. Schwartz, G. Barbin, S. Bischoff, M. Garbarg, H. Pollard, in preparation).
20. W. W. Douglas, in *The Pharmacological Basis of Therapeutics*, C. S. Goodman and A. Gilman, Eds. (Macmillan, London, 1970), p. 621.
21. H. Pollard, S. Bischoff, J. C. Schwartz, *Eur. J. Pharmacol.* **24**, 399 (1973); *J. Pharmacol. Exp. Ther.* **190**, 88 (1974).
22. J. Schultz and J. W. Daly, *J. Biol. Chem.* **248**, 843 (1973).
23. The existence of a large-sized nonneuronal pool of histamine would account for the peculiar developmental pattern of the amine in rat brain. Whereas noradrenaline or serotonin contents increase steadily during ontogenesis, the level of histamine is high at birth (*24*), that is, at a time when most central synapses are not yet formed. In contrast, the activity of L-histidine decarboxylase is low in the newborn, then its development (*24*) strictly parallels the cerebral synaptogenesis [G. K. Aghajanian and F. E. Bloom, *Brain Res.* **6**, 710 (1967)]. This nonneuronal store for histamine, already present at birth and turning over slowly, as indicated by a low ratio of L-histidine decarboxylase activity to the histamine content, would be left intact by the process of Wallerian degeneration. The identity of the cells holding the bulk of histamine in rat brain is a matter of conjecture, but mast cells are likely candidates for this role; although they are known to be rare in the brain (*1*), they could nevertheless contribute markedly to the cerebral amine content owing to their high histamine concentration associated with a low histidine decarboxylase activity [R. W. Schayer, in *Handbook of Experimental Pharmacology*, M. Rocha e Silva, Ed. (Springer, Berlin, 1966), vol. 18, p. 688].
24. J. C. Schwartz, C. Lampart, C. Rose, M. C. Rehault, S. Bischoff, H. Pollard, *J. Neurochem.* **18**, 1787 (1971).
25. We thank E. Pattou for the histological control. Supported by contracts from l'Institut National de la Santé et de la Recherche Médicale (I.N.S.E.R.M.), la D.R.M.E. and la Fondation pour la Recherche Médicale.

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Interferon Induction: Tool for Establishing Interactions among Homopolyribonucleotides

Abstract. *Hitherto unrecognized interactions between homopolyribonucleotides and complexes thereof are suggested by interferon induction data obtained in a highly sensitive assay system of primary rabbit kidney cell cultures superinduced by metabolic inhibitors.*

Interferon induction can be considered a stringent biological test for the double-strandedness of polyribonucleotides, since double-stranded RNA's are markedly more effective as interferon inducers than their single- and triple-stranded counterparts (*1–4*). Primary rabbit kidney cell cultures are among the most sensitive cell types for assaying the interferon-inducing capacity and antiviral activity of double-stranded RNA's (*5, 6*), and their responsiveness is even enhanced when the cells are treated with metabolic inhibitors, such as cycloheximide and actinomycin D, sometime after their exposure to the double-stranded RNA (*7*). This paradoxical enhancement of interferon production by judicious treatment with metabolic inhibitors has been referred to as "superinduction," a term originally coined for the increase of tyrosine aminotransferase in hydrocortisone-induced cells upon actinomycin D treatment (*8*). In this report, we demonstrate that this sensitive assay system can be employed to identify the occurrence of novel polynucleotide interactions and to gain insight into the nature of the resultant product or products.

In primary rabbit kidney cell cultures superinduced with cycloheximide and actinomycin D, the homopolymers (*9*) polyadenylic acid [poly(A)] and polyuridylic acid [poly(U)], on the one hand, and polyinosinic acid [poly(I)] and polycytidylic acid [(poly(C)], on the other hand, proved unable to reverse the interferon-inducing capacity of the respective duplexes poly(A) · poly(U) and poly(I) · poly(C) when the duplexes were applied to the cells 1 hour later (*4*).

It was concluded from these data that single-stranded homopolymers did not prevent the interaction of the active homopolymer duplexes with the postulated (*2*) cellular receptor site (or sites) for interferon induction. However, single homopolymers were not invariably inactive in reducing the interferon-inducing capacity of the active duplexes; depending on the kind of homopolymer that was added first and on the kind of the homopolymer complex that was added second, the interferon-inducing activity of the latter was depressed, enhanced, or unaffected (Table 1). For example, poly(I) and poly(dUz) [poly(2′-azido-2′-deoxyuridylic acid)] (*10, 11*) caused a significant reduction of the interferon-inducing capacity of poly(A) · poly(U) and poly(A) · poly(rT) (polyribothymidylic acid) applied to the cells 1 hour after the homopolymers were applied. However, poly(I) and poly(dUz) did not affect the activity of poly(I) · poly(C). Poly(C) did not influence the interferon-inducing activity of poly(A) · poly(U) or poly(A) · poly(rT) but boosted the interferon response to poly(A) · poly(I) (a triple-stranded polymer) up to the level generally observed for poly(I) · poly(C). The interferon-inducing activity of poly(A) · poly(U) was not only reversed by poly(I) and poly(dUz) but also by analogous homopolymers, such as poly(X) (*12*) and poly(dUf) [poly(2′-fluorodeoxyuridylic acid)] (*13*) (data not shown).

With most systems studied, similar shifts in interferon production were noted regardless of whether the single homopolynucleotide (*a*) was added to the cells before the homopolynucleotide complex (*b*) (that is, $a \rightarrow b$), after ($b \rightarrow a$), or together ($a + b$). These data suggest that both in the test tube and at the cellular level homopolynucleotide complexes, such as poly(A) · poly(U), must be equally susceptible to fundamental structural transitions. That poly(I) may cause such alterations at the cellular level is no surprise, since poly(I) has been shown previously to form an active complex with poly(C) after the polymers had been added to cells separately in an interval of one to several hours (*6*). Although poly(U) reduced the activity of poly(A) · poly(U) when mixed with, or applied to, the cells after poly(A) · poly(U), it failed to do so when applied to the cells before poly(A) · poly(U). In marked contrast, poly(dUz) reversed the activity of poly(A) · poly(U) when it was applied to the cells before the duplex. The differential behavior of poly(U) and poly(dUz) may be accounted for by differences in susceptibility to degradation by nucleases. Poly(U) is very susceptible, whereas poly(dUz) is not (*10*). Poly(U) may have been degraded by cellular nucleases before it could be reached by poly(A) · poly(U).

Several lines of evidence indicate that the shifts in interferon production by the systems depicted in Table 1 originate from a specific interaction between the single homopolynucleotide and the

homopolynucleotide complex: First, changes in interferon-inducing activity were only seen when the single homopolymer was complementary to one of the homopolymers of the complex; second, it did not matter whether the single homopolymer was added to the cells before, after, or mixed with the complex; and, third, the inhibitory effect of the single homopolymer [poly(I), poly(dUz), and so forth] on the interferon-inducing activity of the complex [poly(A) • poly(U) and so forth] was absent when the single homopolymers had been complexed before use, for example, to poly(A) • 2 poly(I), poly(A) • poly(dUz), and so forth (*14*) (data not shown).

The question then arises as to how this specific interaction between the single homopolynucleotide and the homopolynucleotide complex could be visualized. Out of several possibilities the following might be considered: (i) triple-strand formation, (ii) strand displacement, or (iii) strand displacement and triple-strand formation. These may be exemplified for the first system (first item in Table 1) (*15*):

(i) poly(I) + poly(A)·poly(U) → poly(I)·poly(A)·poly(U)

(ii) 2 poly(I) + poly(A)·poly(U) → poly(A)·2 poly(I) + poly(U)

(iii) 2 poly(I) + 2 poly(A)·poly(U) → poly(A)·2 poly(I) + poly(A)· 2 poly(U)

For the second system they are exemplified by the second item in Table 1:

(i) poly(dUz) + poly(A)·poly(U) → poly(dUz)·poly(A)·poly(U)

(ii) poly(dUz) + poly(A)·poly(U) → poly(A)·poly(dUz) + poly(U)

(iii) poly(dUz) + 2 poly(A)·poly(U) → poly(A)·poly(dUz) + poly(A)· 2 poly(U)

All these reactions would result in a significantly reduced interferon production, since their reaction products are known to be inactive as interferon inducers [poly(A) • 2 poly(I) (*3*); poly(A) • 2 poly(U) (*4*); poly(A) • poly(dUz) (*11*)] or anticipated to be ineffective [poly(A) • poly(U) • poly(I), poly(dUz) • poly(A) • poly(U)] because of their triple-stranded structure (*4*).

To distinguish between the possibilities outlined above, the reaction products were analyzed by different physicochemical and biochemical techniques (ultraviolet absorption mixing curves and melting profiles, ribonuclease sensitivity, or sucrose velocity gradient sedimentation, or all). It appears (*14*) that at least with the systems poly(I) + poly(A) • poly(U), poly(dUz) + poly(A) • poly(U), poly(I) + poly(A) • poly(rT), and poly(U) + poly(A) • 2 poly(I) triple-stranded complexes were formed [poly(A) • poly(U) • poly(I), poly(dUz) • poly(A) • poly(U), poly(I) • poly(A) • poly(rT), and poly(A) • poly(U) • poly(I), respectively], complexes which have never been recognized [possibility (i)]. Whether similar triplexes [poly(dUz) • poly(A) • poly(rT), . . .] were also formed in the other systems is not yet known. However, the dramatic increase in interferon inducing activity observed with the system poly(C) + poly(A) • 2 poly(I) can only be explained by a dismutation reaction according to:

2 poly(C) + poly(A)·2 poly(I) → poly(A) + 2 poly(I)·poly(C)

Physicochemical evidence for this reaction has been provided previously (*16*) and has recently been confirmed by sucrose velocity gradient sedimentation studies (*14*).

The sensitive interferon assay described herein may also be utilized in the study of the interactions of self-

Table 1. Interactions among homopolynucleotides and homopolynucleotide complexes as monitored by interferon induction in primary rabbit kidney cell cultures superinduced with cycloheximide and actinomycin D.

System		Interferon titer ratio* for sequence of addition†:			Postulated reaction product
a	*b*	*a* → *b*	*a* + *b*	*b* → *a*	
Poly(I)	Poly(A)•poly(U)	1/30	1/30	1/4	Poly(I)•poly(A)•poly(U)‡
Poly(dUz)	Poly(A)•poly(U)	⩽1/100	⩽1/100	1/5	Poly(dUz)•poly(A)•poly(U)‡
Poly(C)	Poly(A)•poly(U)	1/1			
Poly(A)	Poly(A)•poly(U)	1/1	1/1	1/1	
Poly(U)	Poly(A)•poly(U)	1/1	1/10	1/5	Poly(U)•poly(A)•poly(U)‡
Poly(I)	Poly(A)•poly(rT)	1/200	1/30	1/10	Poly(I)•poly(A)•poly(rT)‡
Poly(dUz)	Poly(A)•poly(rT)	1/30	1/40	1/5	Poly(dUz)•poly(A)•poly(rT)§
Poly(C)	Poly(A)•poly(rT)	1/1			
Poly(A)	Poly(A)•poly(rT)	1/1	1/1	1/1	
Poly(rT)	Poly(A)•poly(rT)	1/5	⩽1/20	1/15	Poly(rT)•poly(A)•poly(rT)§
Poly(U)	Poly(A)•2 poly(I)	1/1	1/1	1/1	Poly(I)•poly(A)•poly(U)‡
Poly(rT)	Poly(A)•2 poly(I)	1/1	5–10	1–2	Poly(I)•poly(A)•poly(rT)§
Poly(C)	Poly(A)•2 poly(I)	⩾3000	⩾3000	⩾3000	Poly(I)•poly(C)‡
Poly(I)	Poly(I)•poly(C)	1/1			
Poly(C)	Poly(I)•poly(C)	1/1			
Poly(A)	Poly(I)•poly(C)	1/1	1/1		
Poly(dUz)	Poly(I)•poly(C)	1/1			
Poly(U)	Poly(A)•poly(dUz)	1/1	1/1	1–2	Poly(dUz)•poly(A)•poly(U)‖
Poly(rT)	Poly(A)•poly(dUz)	1/1		3–6	Poly(dUz)•poly(A)•poly(rT)‖

* Ratio of interferon titer obtained in cell cultures exposed to both *a* (homopolynucleotide) and *b* (homopolynucleotide complex) to interferon titer obtained in cells exposed to *b* only. The interferon titers [titration procedure as described in (*4*)] obtained in cell cultures exposed to *b* alone averaged 1,590 unit/ml (range, 300 to 4,500) for poly(A) · poly(U); 3,120 unit/ml range, 1,000 to 10,000) for poly(A) · poly(rT); 13,360 unit/ml (range, 3,000 to 30,000) for poly(I) · poly(C); but only 3 to 10 unit/ml for poly(A) · 2 poly(I) and poly(A) · poly(dUz). The interferon titers obtained with the single homopolymers (*a*) were also insignificant (3 to 10 unit/ml). These interferon titers correspond well to previously reported data (*3, 4, 11*). They represent mean values for about 10 to 20 experiments. The interferon titer ratios represent the mean values for at least three experiments. † All polymer solutions were made up in Eagle's minimal essential medium (MEM). If the homopolymers (*a*) and homopolymer complexes (*b*) were added to the cell cultures in sequential order (*a* → *b* or *b* → *a*), the cells (in 60-mm Falcon plastic petri dishes) were incubated for 1 hour at 37°C with the first polymer at 10 μg/ml (1 ml per petri dish), washed three times with MEM, and immediately thereafter exposed to the second polymer at 10 μg/ml per petri dish for another hour at 37°C, washed again, and then processed for interferon production (*19*). In another set of experiments (*a* + *b*), the homopolymer (*a*) and homopolymer complex (*b*) were first mixed at final concentrations of 5 and 10 μg/ml, respectively, incubated for 1 hour at 37°C, and then added to the cells (1 ml per petri dish). The cells were then incubated for 1 hour at 37°C, washed three times with MEM, and further processed for interferon production (*19*). ‡ Proved by ultraviolet-mixing curves, ultraviolet-melting profiles, ribonuclease sensitivity experiments, or sucrose velocity gradient sedimentation (or all) (*11*). § Assumed in analogy with the corresponding poly(U) triplexes. ‖ Still hypothetical; formation of these triplexes compatible with interferon induction data.

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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References and Notes

1. A. K. Field, A. A. Tytell, G. P. Lampson, M. R. Hilleman, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1004 (1967).
2. C. Colby and M. J. Chamberlin, *ibid.* **63**, 160 (1969).
3. E. De Clercq and T. C. Merigan, *Nature (Lond.)* **222**, 1148 (1969); E. De Clercq, F. Eckstein, T. C. Merigan, *Ann. N.Y. Acad. Sci.* **173**, 444 (1970).
4. E. De Clercq, P. F. Torrence, B. Witkop, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 182 (1974).
5. A. K. Field, A. A. Tytell, G. P. Lampson, M. R. Hilleman, *ibid.* **61**, 340 (1968); E. De Clercq and P. De Somer, *J. Gen. Virol.* **19**, 113 (1973).
6. E. De Clercq and P. De Somer, *Science* **173**, 260 (1971); *J. Virol.* **9**, 721 (1972).
7. J. Vilcek, T. G. Rossman, F. Varacalli, *Nature (Lond.)* **222**, 682 (1969); Y. H. Tan, J. A. Armstrong, Y. H. Ke, M. Ho, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 464 (1970); Y. H. Tan, J. A. Armstrong, M. Ho, *Virology* **44**, 503 (1971); J. Vilcek and M. H. Ng, *J. Virol.* **7**, 588 (1971).
8. L. D. Garren, R. R. Howell, G. M. Tomkins, R. M. Crocco, *Proc. Natl. Acad. Sci. U.S.A.* **52**, 1121 (1964); G. M. Tomkins, T. D. Gelehrter, D. Granner, D. Martin, Jr., H. H. Samuels, E. B. Thompson, *Science* **166**, 1474 (1969).
9. Origin and characteristics of the polyribonucleotide preparations used are for poly(I), P-L Biochemicals (S_{20}, 9.0, 9.4) or Miles Laboratories (S_{20}, 4.8); for poly(C), P-L Biochemicals (S_{20}, 10.0, 13.7) or Miles Laboratories (S_{20}, 3.6, 4.3, 5.7); for poly(A), Miles Laboratories (S_{20}, 7.2, 8.0, 9.2); for poly(U), Miles Laboratories (S_{20}, 3.5, 4.9, 6.2, 7.4); for poly(rT), see (*4*) (S_{20}, 6.9); for poly(dUz); see (*10*) (S_{20}, 8.0, 12.6).
10. P. F. Torrence, A. M. Bobst, J. A. Waters, B. Witkop, *Biochemistry* **12**, 3962 (1973).
11. P. F. Torrence, J. A. Waters, C. E. Buckler, B. Witkop, *Biochem. Biophys. Res. Commun.* **52**, 890 (1973).
12. P. F. Torrence, E. De Clercq, B. Witkop, in preparation.
13. B. Janik, M. P. Kotick, T. H. Kreiser, L. F. Reverman, R. G. Sommer, D. P. Wilson, *Biochem. Biophys. Res. Commun.* **46**, 1153 (1972); E. De Clercq and B. Janik, *Biochim. Biophys. Acta* **324**, 50 (1973); E. De Clercq and B. Janik, unpublished data.
14. E. De Clercq, P. F. Torrence, B. Witkop, in preparation.
15. The complex between poly(I) and poly(A) is presented as poly(A)·2 poly(I) according to A. Rich, *Nature (Lond.)* **181**, 521 (1957).
16. P. B. Sigler, D. R. Davies, H. T. Miles, *J. Mol. Biol.* **5**, 709 (1962).
17. S. Arnott and P. J. Bond, *Nat. New Biol.* **244**, 99 (1973).
18. F. Crick, *Nature (Lond.)* **234**, 25 (1971).
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.