The ALA synthetase in rat liver has a half-life of about 70 minutes (*1*). It is possible that glucagon achieves its rapid low-amplitude induction by promoting conditions which retard the rapid destruction of already present enzyme.

J. A. SIMONS

Department of Biology,
College of William and Mary,
Williamsburg, Virginia 23185

References

1. S. Granick and G. Urata, *J. Biol. Chem.* **238**, 821 (1963).
2. S. Granick, *ibid.* **241**, 1358 (1966).
3. D. P. Tschudy, M. G. Perlroth, H. S. Marver, A. Collins, G. Hunter, Jr., M. Rechcigl, Jr., *Proc. Nat. Acad. Sci. U.S.* **53**, 841 (1965).
4. F. H. Welland, E. S. Hellman, E. M. Gaddis, A. Collins, G. W. Hunter, D. P. Tschudy, *Metabolism* **13**, 232 (1964).
5. H. S. Marver, A. Collins, D. P. Tschudy, M. Rechcigl, Jr., *J. Biol. Chem.* **241**, 4323 (1966).
6. H. S. Marver, D. P. Tschudy, M. G. Perlroth, A. Collins, *ibid.*, p. 2803.
7. O. Greengard, *Science* **163**, 891 (1969).
8. J. A. Simons and E. J. Boell, *Amer. Zool.* **7**, 748 (1967).
9. A. Kappas, C. S. Song, R. D. Levere, R. A. Sachson, S. Granick, *Proc. Nat. Acad. Sci. U.S.* **61**, 509 (1968).
10. A. Waxman, A. Collins, D. P. Tschudy, *Biochem. Biophys. Res. Commun.* **24**, 675 (1966).
11. R. W. Butcher, G. A. Robinson, J. G. Hardman, E. W. Sutherland, *Advan. Enzyme Regul. Proc. Symp. Regul. Enzyme Activ. Syn. Norm. Neoplast. Tissues* **6**, 357 (1968).

21 November 1969; revised 15 January 1970

Polyinosinic Acid • Polycytidylic Acid: Inhibition of DNA Synthesis Stimulated by Isoproterenol

Abstract. *Polyinosinic acid • polycytidylic acid (poly I • poly C) inhibits isoproterenol-stimulated DNA synthesis in salivary glands of mice. A single intraperitoneal injection of 250 micrograms of poly I·poly C inhibits the stimulation of DNA synthesis when given 10 minutes before isoproterenol or at any time during the 20-hour lag period between the injection of isoproterenol and the subsequent DNA synthesis. The inhibition caused by poly I·poly C is not due to a generalized toxic action but seems to be exerted through a relatively selective mechanism. Polyuridylic acid and diethylaminoethyl dextran are less effective in inhibiting isoproterenol-stimulated DNA synthesis.*

The synthetic double-stranded RNA, polyinosinic acid • polycytidylic acid, poly I • poly C induces synthesis of interferon in mammalian cells (*1*) and inhibits the growth of certain virus-induced sarcomas and leukemias in mice, as well as of some other tumors not known to contain infectious oncogenic viruses (*2, 3*). Since these results did not conclusively show that the mechanism of action of poly I • poly C was antiviral, we investigated its effect on the stimulation of DNA synthesis and cell division that is caused in salivary glands of mice by isoproterenol (IPR), a synthetic catecholamine (*4*). Both poly I • poly C and the ammonium salt of polyuridylic acid (poly U) (*5*) were stored at 4°C; they were dissolved shortly before use in distilled-deionized water. Diethylaminoethyl (DEAE) dextran was stored at room temperature. Isoproterenol [2-(3,4-dihydroxyphenyl)2-isopropylaminoethanol hydrochloride] was dissolved in water at a concentration of 4.0 mg/0.2 ml immediately before intraperitoneal injection into male Swiss mice weighing about 30 g. [^{3}H]Thymidine (10 μc) was injected subcutaneously into each mouse 26 hours after the administration of IPR (the peak of DNA synthesis in mice) (*6*), and the animals were killed 30 minutes later by cervical dislocation. The salivary glands were quickly dissected from adhering fat and lymph nodes. The three major salivary glands (submaxillary, sublingual, and parotid) from each mouse were pooled and homogenized in 0.25*M* sucrose containing 5 percent citric acid. The homogenates were extracted by the method of Scott *et al.* as modified by Hinrichs *et al.* (*7*), to obtain the specific activity of salivary gland DNA.

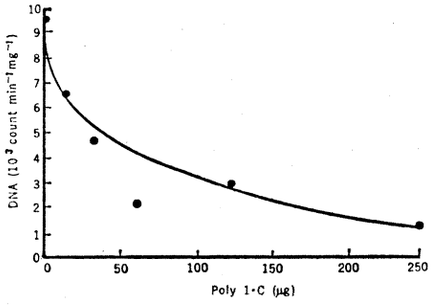

Fig. 1. The effect of increasing amounts of poly I • poly C on isoproterenol-stimulated DNA synthesis in salivary glands of mice. Poly I • poly C was given 10 minutes before isoproterenol, and the specific activity of salivary gland DNA was determined 26.5 hours after injection of isoproterenol and 30 minutes after an injection of tritiated thymidine.

The effect of a single intraperitoneal injection of 250 μg of poly I • poly C, poly U, or DEAE dextran 10 minutes before the injection of IPR on DNA synthesis in mouse salivary glands is shown in Table 1. Whereas DEAE dextran and poly U have only a moderate effect, poly I • poly C almost completely inhibits the stimulation of DNA synthesis caused by IPR in the salivary glands of mice.

The inhibition of IPR-stimulated DNA synthesis by poly I · poly C is dependent on the dose used (Fig. 1). As little as 15 μg of poly I · poly C produced a 30-percent inhibition of DNA synthesis.

When poly I · poly C (250 μg) was injected at the intervals, before or after IPR as indicated in Fig. 2, and DNA synthesis was tested 26 hours after IPR was administered, the stimulation of DNA synthesis was inhibited, under these conditions, by a single intraperitoneal injection of poly I · poly C from 10 minutes before until 18 hours after injection of IPR. After DNA synthesis began (about 20 hours after IPR), the injection of poly I · poly C was without effect. Neither poly U nor DEAE dextran caused an inhibition of DNA synthesis at any time after IPR (not shown).

It has been reported that poly I · poly C has some toxic effects on rabbits (*8*). The fact that poly I · poly C does not interfere with DNA synthesis once synthesis has begun seems to rule out a generalized toxic effect. This hypothesis was confirmed by the following experiment. A single injection of IPR causes a marked decrease in the α-amylase activity of salivary glands of rats

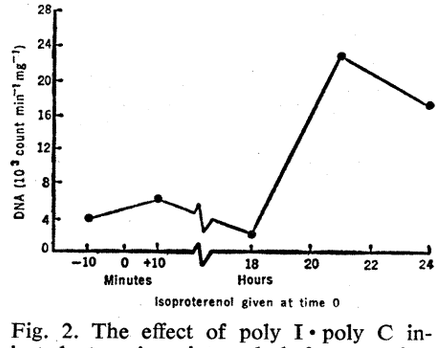

Fig. 2. The effect of poly I • poly C injected at various intervals before or after isoproterenol, on isoproterenol-stimulated DNA synthesis in mouse salivary glands. On the abscissa is the time before or after isoproterenol at which poly I • poly C was injected. On the ordinate is the specific activity of salivary gland DNA as determined 26.5 hours after injection of isoproterenol and 30 minutes after an injection of tritiated thymidine.

Table 1. Effect of poly I • poly C on isoproterenol-stimulated DNA synthesis. Poly I • poly C, poly U, or DEAE dextran (250 μg doses) was injected intraperitoneally 10 minutes before isoproterenol (IPR), 4 mg intraperitoneally; tritiated thymidine was given subcutaneously (10 μc to each mouse) 26 hours after IPR; the animals were killed 30 minutes later (number of mice is shown in parentheses). The specific activity of salivary gland DNA is expressed in counts per minute per milligram of DNA (± standard deviation).

Treatment	Specific activity of salivary gland DNA
None (9)	2,457 ± 1021
IPR only (8)	21,080 ± 4028
Poly I • poly C + IPR (6)	3,081 ± 1600
Poly U + IPR (6)	11,695 ± 1046
DEAE-dextran + IPR (6)	12,058 ± 6400

Table 2. Effect of poly I • poly C on the synthesis of α-amylase in mouse salivary gland. All animals, except controls, received 4 mg of isoproterenol (IPR) intraperitoneally. Poly I • poly C (250 μg per mouse) was given 2 hours after IPR. α-Amylase activity (units of amylase per milligram of protein) was determined by the method of Bernfeld (*12*). Three to six animals per group (mean ± standard deviation).

Time after IPR (hr)	Treatment	Amylase
	None	12.6 ± 1.8
2	IPR only	1.7 ± 0.1
18	IPR only	8.2 ± 0.3
24	IPR only	9.4 ± 1.1
18	IPR + poly I • poly C	7.3 ± 0.7
24	IPR + poly I • poly C	7.5 ± 0.2

and mice (*9*). The enzyme is then resynthesized and, within 24 hours after IPR, its activity has returned to values just below those of control animals (*9*). Table 2 indicates that poly I · poly C, injected 2 hours after IPR (at the time of minimum α-amylase activity), has little effect on the reappearance of α-amylase activity at later times.

Gresser *et al.* (*3*) have demonstrated that survival of mice inoculated with tumor cells of viral etiology increases when the mice are treated with interferon preparations. They also showed that interferon preparations delayed the evolution of Friend and Rauscher leukemias in mice (*10, 11*) and stated that, although a direct action of interferon on the proliferation of viral-infected transformed cells could not be excluded, the antitumor effect of interferon was probably due to its antiviral property. Levy *et al.* (*11*) have shown that poly I • poly C inhibited the growth of a reticulum cell sarcoma, a lymphatic lymphoma, and a fibrosarcoma which apparently do not contain infectious oncogenic viruses. They postulated that the action of poly I · poly C could be due to (i) enhanced immunological rejection of foreign antigens; or (ii) a direct action on tumor such as an induction of modified ribosomes able to make even finer distinctions than those made by interferon-type ribosomes; or (iii) the possibility that poly I · poly C produces changes in the blood supply to the tumor with resulting ischemic necrosis. The results of our experiments cannot be explained in terms of a generalized toxic effect, and it is difficult to invoke an immunological mechanism in the inhibition of IPR-stimulated DNA synthesis. As to the antiviral action of poly I · poly C, one should consider the possibility that IPR induces the replication of a latent DNA-containing virus and that the development of this virus is blocked by poly I · poly C. However, the effect of IPR is a bona fide stimulation of cellular proliferation (*4, 6*), characterized by duplication of the amount of cellular DNA and by mitosis. That mitosis in mammalian cells may be regulated by a latent virus is a still unsupported possibility. Finally, the inhibitory effect of poly I · poly C on IPR-stimulated DNA synthesis resembles closely that of low doses of actinomycin D (*6*), and it is possible that it may exert an effect on the transcription of the cell genome.

FREDERIC T. SEROTA
RENATO BASERGA
Department of Pathology and *Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania 19140*

References and Notes

→ A. K. Field, A. A. Tytell, G. P. Lampson, M. R. Hilleman, *Proc. Nat. Acad. Sci. U.S.* **58**, 1004 (1967).
→ P. S. Sarma, G. Shiu, R. H. Neubauer, S. Baron, R. J. Huebner, *ibid.* **62**, 1046 (1969); J. K. Youn, G. Barski, J. Huppert, *C. R. Acad. Sci. Paris* **267**, 816 (1968); I. Gresser, C. Bourali, J. P. Levy, D. Fontaine-Brouty-Boyé, M. T. Thomas, *ibid.* **268**, 994 (1969).
→ I. Gresser, C. Bourali, J. P. Levy, D. Fontaine-Brouty-Boyé, M. T. Thomas, *Proc. Nat. Acad. Sci. U.S.* **63**, 51 (1969).
4. R. Baserga, *Life Sci.* **5**, 2033 (1966).
5. Poly I • poly C and poly U (ammonium salt) were from Miles Laboratories, Inc., Elkhart, Indiana; DEAE dextran, from Pharmacia Co., Upsala, Sweden; [methyl-H^3]thymidine (18.3 c/mmole), from Amersham/Searle Co., Des Plaines, Illinois; isoproterenol, from Winthrop Laboratories, New York.
6. R. Baserga and S. Heffler, *Exp. Cell Res.* **46**, 571 (1967).
7. J. F. Scott, A. P. Fraccastoro, E. B. Taft, *J. Histochem. Cytochem.* **4**, 1 (1956); H. R. Hinrichs, R. O. Petersen, R. Baserga, *Arch. Pathol.* **78**, 245 (1964).
8. M. Absher and W. R. Stinebring, *Nature* **223**, 715 (1969); H. L. Lindsay, P. W. Trown, J. Brandt, M. Forbes, *ibid.*, p. 717; R. H. Adamson and S. Fabro, *ibid.*, p. 718.
9. P. Byrt, *ibid.* **212**, 1212 (1966); J. P. Whitlock, Jr., R. Kaufman, R. Baserga, *Cancer Res.* **28**, 2211 (1968).
10. I. Gresser, J. Coppey, E. Falcoff, D. Fontaine, C. R. *Acad. Sci. Paris,* **263**, 586 (1966); I. Gresser, L. Berman, G. De-Thé, D. Brouty-Boyé, J. Coppey, E. Falcoff, *J. Nat. Cancer Inst.* **41**, 505 (1968).
→ H. B. Levy, L. W. Law, A. S. Rabson, *Proc. Nat. Acad. Sci. U.S.* **62**, 357 (1969).
12. P. Bernfeld, *Meth. Enzymol.* **1**, 149 (1955).

13. This work was supported by PHS research grant DE-02678.

13 November 1969; revised 31 December 1969

Chromosome Number Variation in a Stick Insect Didymuria violescens (Leach)

Abstract. *Seven major races, with diploid numbers ranging from 26 to 40, and three types of sex-chromosome mechanism were found in the Australian phasmatid* Didymuria violescens *(Leach). The differences between chromosome complements are mainly due to translocations between autosomes, and to translocations between autosomes and sex chromosomes. The geographic pattern of chromosome variation and the characteristics of hybrids implicate chromosomal rearrangements in mechanisms of speciation.*

I have found extensive chromosomal variation in *Didymuria violescens* (Leach). This phasmatid is endemic to Australia and is distributed in the southeastern region of the continent. It occurs in the sclerophyllous Eucalypt forests of the coast and adjacent highlands, extending from southern Queensland south to Victoria, then westward to the Mount Lofty Ranges in South Australia. Within this area, seven races differing in chromosome number have been discovered (Fig. 1). The distributions of these are often contiguous and exhibit a mosaic pattern which was constant in successive seasons. The seven races possess distinct karyotypes, but they appear morphologically indistinguishable. On the available evidence (*1*), all would be referred to one species. These insects are generally sedentary. The adult males are capable of limited flight, but the females have very small wings and cannot fly. Dispersal is slow and is restricted to movement between trees either through the canopy or on the ground.

Diploid chromosome number varies from 40 to 26 in females. Four races have the XO(male):XX(female) sex-chromosome mechanism that is characteristic of phasmatids (*2*). These are the 39 male:40 female race of predominantly coastal distribution, the 37:38 race of the central eastern highlands of New South Wales, the 35:36 race of the southern highlands (New