Table 1. Dependence of phagocytoxic effect on presence of serum.

Additions	Percent of bacteria killed	
	Serum	No serum
None	70.1	42.8
Cysteine (2.5 μmole/ml)	63.7	37.3
Whole smoke (6 ml)	1.2	49.0
Whole smoke (6 ml) + cysteine (2.5 μmole/ml)	60.7	47.0

at a concentration of 0.025 micromole per milliliter, and was somewhat variable at intermediate concentrations. Cysteine also prevented the phagocytoxic effect of cigarette smoke, in concentrations of 2.5 micromoles per milliliter, but not at 0.25 micromole per milliliter (Fig. 2). There was a direct dose-response effect between 0.3 and 2.5 micromoles per milliliter. Calculations showed that between 0.2 and 0.4 micromole of cysteine and perhaps less of glutathione was required per milliliter of cigarette smoke for the protective effect. The effect could be overcome by the addition of larger amounts of cigarette smoke; glutathione and cysteine seemed to be titrating some factor in the smoke.

Glutathione and cysteine are sulfhydryl-containing reducing agents and biologic antioxidants. The disulfide forms of these compounds showed no significant protective activity for the macrophages against the smoke. Potassium ferrocyanide and ascorbic acid also failed to protect the macrophages. In fact, ascorbic acid reduced the protective efficacy of glutathione when both substances were added together.

Cigarette smoke produced a rapid change in oxidation-reduction potential when bubbled through an aqueous medium in a closed chamber, but this effect was not found consistently in the flask system. Furthermore, such changes in redox potential per se did not affect phagocytic activity.

Finally (Table 1), it was observed that serum protein was needed to obtain both the toxic effect of cigarette smoke and the protective action of glutathione. In the absence of serum, phagocytic activity was reduced, cigarette smoke was nontoxic, and cysteine was inactive. As little as 0.5 percent serum evoked these effects, and dialyzed serum was as effective as whole serum. The role of the protein might be (i) to improve binding of the toxic material, (ii) to transport the toxic substances, (iii) to stimulate phagocytosis, or (iv) to exert a more complex action on cell membrane. Binding is an unlikely explanation, since the toxic substance is equally well absorbed into aqueous and protein solutions, and is readily dialyzed out of protein solutions. Stimulation of phagocytosis is clearly shown by the data to be a factor. An effect at the cell membrane is suggested by the observation that coincident with the loss of phagocytic activity, cells exposed to cigarette smoke separated from the flask surface, although they did not lose their ability to exclude vital dyes. Cell separation is prevented by glutathione and cysteine.

A recent report by Thomas *et al.* (*2*), showing lipoperoxidation of lung lipids in rats exposed to nitrogen dioxide, and partial prevention of the effect by α-tocopherol, may be relevant to the suggested mechanism of action on cell membrane. Similar findings for other oxidant gases and air pollutants would permit a unifying concept to be advanced to explain the damaging action of a broad group of agents in the causation of so-called nonspecific disease in the lungs.

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References and Notes

1. G. M. Green and D. Carolin, *New Eng. J. Med.* **276**, 421 (1967).
2. H. V. Thomas, P. K. Miller, R. L. Lyman, *Science* **159**, 532 (1968).
3. Supported by grants from the American Medical Association Research and Educational Foundation and the National Institute of Allergy and Infectious Diseases (A1-06577). Formerly Edward Livingston Trudeau Fellow of the American Thoracic Society.
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Herpetic Keratoconjunctivitis: Therapy with Synthetic Double-Stranded RNA

Abstract. *A study was undertaken in rabbits to determine how late in the course of keratoconjunctivitis caused by herpes simplex recovery could be effected by an inducer of interferon. Interferon was induced by means of synthetic double-stranded RNA copolymer formed with polynosinic acid : polycytidilic acid RNA. Therapy promotes recovery from severe and fully established keratoconjunctivitis for which treatment was begun as late as 3 days after virus inoculation. No drug toxicity was observed in the therapeutic dose range. These findings further support the proposed role of the interferon mechanism in the natural recovery of already established viral infection. They also suggest the usefulness of interferon inducers in viral infections of man.*

Considerable evidence has been developed to link interferon with natural recovery from already established viral infections (*1–5*). To further substantiate this concept, it is necessary to demonstrate that interferon, in excess of the amount produced by the body during infection, can enhance recovery from fully established viral infections (*3*). This has not yet been achieved, perhaps because it has not yet been possible to apply concentrations of interferon equal to or in excess of those produced by the body during infection (*4*).

Several synthetic and natural substances such as pyran copolymer (*6*), statolon (*7*), mannans (*8*), endotoxin (*9*), RNA polynucleotides (*10*, *11*), and cycloheximide (*9*) have been shown to induce interferon. These inducers could overcome the difficulty in obtaining sufficient quantities of exogenous interferon (*4*), and might also overcome the problem of species specificity in antiviral uses for interferon. These agents, then, provide the opportunity to study the therapeutic efficacy of the interferon mechanism against already established viral infections. The present experiment was undertaken to examine the ability of high concentrations of interferon induced by double-stranded RNA to enhance recovery from an already established herpetic keratoconjunctivitis in the rabbit eye.

Herpes simplex virus strain 11123, which was originally isolated from recurrent herpes labialis, was used (*12*). Passage 17 times in primary rabbit kidney cultures resulted in a titer of $10^{7.7}$ plaque-forming units (PFU) per milliliter in rabbit kidney cultures. Virus was stored in a mechanical freezer at −70°C. Albino New Zealand rabbits weighing 1.5 to 2 kg were used throughout the experiments. The corneas of anesthetized rabbits were scratched with a 20-gauge needle to produce two horizontal and two vertical lines each measuring approximately 5 mm in length and 0.5 mm in depth. One-tenth milliliter of a suspension containing from

$10^{3.4}$ to $10^{4.4}$ PFU of herpes simplex virus was administered into the lower cul-de-sac of each eye and the lids were closed and rubbed against the eye for 30 seconds.

Twenty-four hours after inoculation, each eye was examined with the biomicroscope, after staining the corneas with fluorescein strips. Grades 0 to 4 were used to grade keratoconjunctivitis: grade 0 is an essentially normal appearing eye; grade 1 is one with slight conjunctival hyperemia and minimal keratitis; grade 2, one with moderate conjunctival hyperemia and slight to moderate keratitis with slight corneal ulceration; grade 3, one with moderately severe conjunctivitis with exudate and corneal ulceration and edema; and grade 4, one with severe conjunctivitis, marked exudation, severe keratitis, corneal ulcer, and edema. The points plotted on the graphs represent the average of the lesion score of at least four eyes.

Synthetic homopolymers of polyinosinic acid and polycytidilic acid were mixed in equimolar concentration in 0.01*M* phosphate-buffered saline at *p*H 7.2 containing $5 \times 10^{-3}M$ MgCl. Formation of double-stranded polyinosinic: polycytidilic acid duplexes (PI:C) (*13*) was evidenced by a 45-percent decrease in optical density as measured at 245 nm. Rabbit interferon was assayed by the inhibition of yield of vesicular stomatitis virus in primary rabbit kidney culture (*14*) or by the cytopathic effect–protection method (*11*).

To evaluate possible irritation and toxicity of the PI:C, two drops of each of two concentrations of PI:C (2000 μg/ml and 200 μg/ml) were instilled three times per day for 3 weeks on intact, as well as scratched, corneas. Epithelial healing of the scratched corneas progressed as rapidly as placebo-treated controls. None of the four rabbit eyes used per dilution of PI:C showed signs of hyperemia, edema, or corneal opacity at repeated biomicroscopic examination over a 21-day period. During the subsequent topical and systemic therapy of herpes simplex keratoconjunctivitis with this inducer, drug toxicity was not detectable. Additional toxicity studies included the single injection of 0.05 ml of PI:C (1000 μg/ml) into the anterior chamber. Repeated biomicroscopic examination for 3 weeks revealed no signs of irritation.

The animals infected with $10^{4.4}$ PFU of herpes simplex virus as described above were subdivided into groups. One group of two rabbits (four eyes) was treated topically with 0.1 ml PI:C (1000 μg/ml), three times daily, beginning 3 hours after inoculation. In other groups of two animals, treatment was begun 1 to 4 days after inoculation. In all groups, treatment was continued for 6 days. Inoculated controls were similarly treated wtih Earle's balanced salt solution. The results of the experiment are shown in Fig. 1. Rabbits that were treated beginning 3 hours after inoculation and 24 hours after inoculation developed minimal keratoconjunctivitis, and their lesions cleared by the 6th day of treatment. Rabbits in which treatment began 2 or 3 days after inoculation had already developed moderate to severe keratoconjunctivitis at the time of initiation of treatment. Keratoconjunctivitis in these rabbits subsided during 4 to 5 days of treatment with PI:C. No therapeutic effect was observed in rabbits in which treatment was initiated 4 days after inoculation. Grade 4 keratoconjunctivitis developed in all control animals.

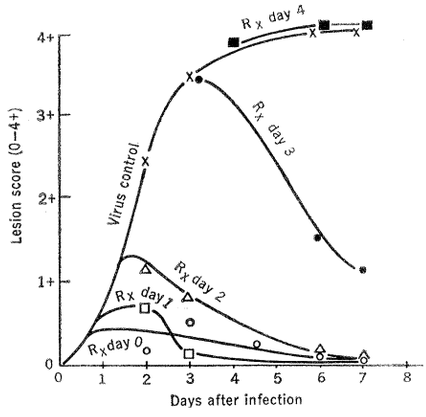

Fig. 1. Response of herpetic keratoconjunctivitis to topical treatment with polyinosinic : polycytidilic acid; R_x indicates treatment.

Similar experiments were carried out to determine the therapeutic effectiveness of PI:C injected into the anterior chamber. Rabbit eyes were infected with $10^{3.4}$ PFU of herpes simplex virus, and each group of two rabbits (four eyes) were injected once with 100 μg of PI:C into the anterior chamber. It could not be excluded that some of the PI:C injected into the anterior chamber leaked through the needle tract into the conjunctival sac. Treatment was given 3 hours after inoculation in one group, and 1 to 3 days after inoculation in the other groups; the findings are shown in Fig. 2. The results were similar to topical treatment in that rapid healing occurred in all rabbits treated as late as day 3. Again, even moderately severe lesions that were present by days 2 and 3 responded to PI:C. Observation on the 13th day after inoculation showed almost complete healing of the treated eyes.

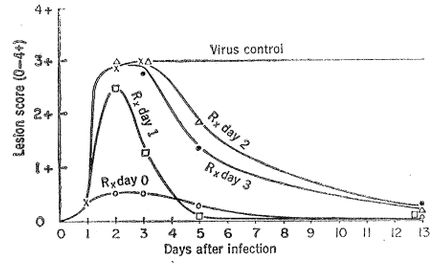

Fig. 2. Response of herpetic keratoconjunctivitis to intracameral polyinosinic : polycytidilic acid; R_x is treatment.

Additional experiments were designed to correlate the therapeutic effect of intravenous PI:C and the degree of induced circulating interferon. Twenty rabbit eyes were inoculated with $10^{3.4}$ PFU herpes simplex virus. Four eyes served as controls. As in previous experiments, therapy was begun at varying times after inoculation as shown in Fig. 3. A single daily dose of 1000 μg of PI:C was given intravenously for six consecutive days. Treatment begun as late as day 3 was followed by a definite therapeutic effect (see Fig. 3). Circulating interferon levels were determined in serum collected daily from two rabbits 3 hours after daily intravenous injections of PI:C for 6 days. The interferon levels on days 1 and 2 were 13,000 unit/ml, but after the sixth dose the circulating interferon level reached only 800 unit/ml. Interferon was not detectable in the serum of untreated animals. The results show very high induction of interferon initially and also demonstrate that partial refractoriness develops to the continued administration of PI:C. This refractoriness has been observed with other inducers of interferon (*15*) and we have confirmed this for intravenous doses of 100, 10, and 1 μg of PI:C.

Interferon exerts its greatest effect prophylactically (*1, 3, 5*). The present findings demonstrate that the interferon mechanism can be applied to enhance recovery from a fully established acute viral infection. Previous studies have shown some protective action of interferon applied after inoculation of virus but before onset of disease (*4, 5*). Re-

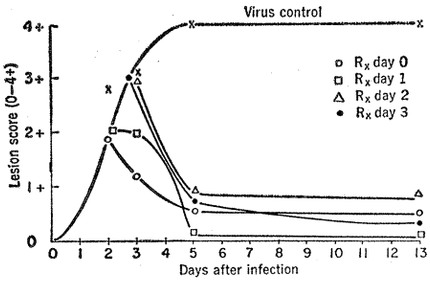

Fig. 3. Response of herpetic keratoconjunctivitis to intravenous polyinosinic:polycytidilic acid; R_x is treatment.

peated administration shortly after onset of virus-induced murine leukemia retarded progression of the disease but was not curative (*16*). Intraocular injection of endotoxin to induce interferon was protective prophylactically (*17*, *18*) and may have resulted in a minimal therapeutic effect, although other effects of endotoxin may have also been involved (*18*). In this study, as well, it is possible that herpes infection resulted in low-level production of interferon (*11*). It had previously been proposed that application of amounts of interferon greater than those that were available at that time would lead to effective therapy (*1–3*, *5*, *19*). The levels and duration of circulating interferon observed in the present study are unusually high (*14*).

Extension of our therapeutic findings to other virus infections is necessary to establish conclusively the therapeutic role of interferon. Such demonstrations seem plausible because herpes simplex virus is only moderately sensitive to the antiviral action of interferon in rabbit cells (*20*). Since there are many viruses that are more sensitive to the antiviral effect of interferon, it seems possible that effective therapy of these infections will be demonstrated.

The therapeutic effect of interferon inducers in established herpetic infection of the rabbit eye raises the possibility of similar application to herpetic infections of the human eye. In inducing viral resistance in human fibroblasts, PI:C is highly active (*21*, *22*). This animal model has been successfully employed in the development of iododeoxyuridine therapy of superficial herpetic infections of the human eye (*23*). Although iododeoxyuridine is an excellent therapeutic agent, it has certain limitations because of its inactivity in nonsurface infections of herpes simplex virus and the existence of drug-resistant strains of virus. Furthermore, it has a narrow antiviral range. Therefore, it seems reasonable to expect that inducers of the interferon system may find application in human eye infections.

Evidence that the therapeutic effectiveness of PI:C is largely attributable to induction of the interferon mechanism comes from the previous (*11*) and present demonstration of high levels of circulating interferon in rabbits receiving this inducer, and the demonstration of strong activation of the interferon mechanism in four different rabbit tissue cultures (kidney, spleen, thymus, and embryo) treated with PI:C (*24*).

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References and Notes

1. A. Issacs, *Advan. Virus Res.* **10**, 1 (1963).
2. S. Baron and H. B. Levy, *Annu. Rev. Microbiol.* **20**, 291 (1966).
3. S. Baron, in *Interferons*, N. B. Finter, Ed. (North-Holland, Amsterdam, 1966), p. 268.
4. S. Baron, C. E. Buckler, C. E. McClosky, R. L. Kirschstein, *J. Immunol.* **96**, 12 (1966); K. Cantell and V. Tomila, *Lancet* **1960-II**, 682 (1960).
5. N. B. Finter, in *Interferons*, N. B. Finter, Ed. (North-Holland, Amsterdam, 1966), p. 232.
6. W. Regelson, in *The Reticuloendothelial System and Atherosclerosis*, N. R. DiLuzio and R. Paoletti, Eds. (Plenum, New York, 1967), pp. 315–332.
7. W. J. Kleinschmidt, J. C. Cline, E. B. Murphy, *Proc. Nat. Acad. Sci. U.S.* **52**, 741 (1964).
8. L. Borecky, V. Lakaric, D. Blaskovic, L. Masler, D. Siki, *Acta Virol.* **2**, 264 (1967).
9. J. S. Youngner, W. R. Stinebring, E. S. Taube, *Virology* **27**, 541 (1965).
10. A. Issacs, R. A. Cox, Z. Rotem, *Lancet* **1963-II**, 113 (1963).
11. A. K. Field, A. A. Tytell, G. P. Lampson, M. R. Hilleman, *Proc. Nat. Acad. Sci. U.S.* **58**, 1004 (1967).
12. Supplied by W. Ashe, NIH.
13. Supplied by H. B. Levy, NIH.
14. N. B. Finter, in *Interferons*, N. B. Finter, Ed. (North-Holland, Amsterdam, 1966), p. 87.
15. J. Vilcek and B. Roda, *Acta Virol.* **6**, 9 (1962).
16. I. Gresser, J. Copey, D. Fontaine-Bruoty-Boye, R. Falcoff, E. Falcoff, F. Zajdela, *Nature* **215**, 174 (1967).
17. J. O. Oh and E. J. Gill, *J. Bact.* **91**, 251 (1966); K. Cantell and V. Tomila, *Lancet* **1960-II**, 682 (1960).
18. R. Polikoff, personal communications (1968).
19. S. Baron, in *Modern Trends in Medical Virology*, R. B. Heath and A. P. Waterson, Eds. (Butterworth, London, 1967), p. 77.
20. A. Billiau and S. Baron, personal communications (1968).
21. M. S. Finklestein, G. Bausek, T. Merigan, personal communications (1968).
22. C. E. Buckler and S. Baron, personal communications (1968).
23. H. E. Kaufman, A. B. Nesburn, E. D. Maloney, *Arch. Ophthalmol.* **67**, 583 (1962).
24. S. Baron, N. Bogomolova, A. G. Billiau, C. E. Buckler, personal communication.
25. Aided by contract PH–43–68–1266 from PHS.

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Airborne Pheromones

Whitten, Bronson, and Greenstein (*1*) described an estrus-inducing pheromone of male mice which is transported by the movement of air currents. Medieval Europeans believed that female birds responded to similar airborne stimuli. The 12th-century *Bestiary*, translated by T. H. White (*2*) has this to say of Perdix, the partridge:

"Desire torments the females so much that even if a wind blows toward them from the males they become pregnant by the smell."

This belief can be traced to the writings of Aristole (*3*) who described unfertilized eggs as *hypenemia* or zephyria.

"They are mistaken who say that hypenemia (barren eggs) are the remains of former acts of sexual intercourse; for young birds, as fowls and geese have been frequently observed to lay such eggs without any sexual intercourse.—The hypenemia are by some persons called zephyria, because they say that birds receive these winds in the spring."

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References

1. W. K. Whitten, F. H. Bronson, J. A. Greenstein, *Science* **161**, 584 (1968).
2. T. H. White, *The Bestiary, A Book of Beasts* (Capricorn Books, Putnam, New York, 1960), p. 137.
3. Aristotle, *Historia Animalium*, R. Cresswell, transl. (Bell, London, 1897), book VI, chap. 2, sec. 5 and 6.

16 September 1968

Obsidian Dating Revisited

In their recent article, Meighan *et al.* (*1*) have reported on the use of obsidian dating as applied to West Mexican archeology. In their report, they use a linear rate of hydration (years/micron). In a footnote they comment that we do not agree with this linear hydration rate on theoretical grounds. We wish to point out that our disagreement is based not on theoretical grounds, but on experimental determination of hydration rate (*2*) as well as on an empirical approach similar to that used by Meighan *et al.*

Other archeologists working in various parts of the world (*3*) have also found that the obsidian hydration follows the relation (thickness)^2 = k time (microns^2/yr).