Table 2. Percentage distribution of radioactivity after reaction of D-adenosine-5′-phosphorimidazolide with a racemic mixture of D-adenosine-8-C^{14} and L-adenosine.

Reaction	A_D	A_DpA_D	$A_DpA_DpA_D$
1	45	51	4
2	73	26	1.1

adenosine under appropriate conditions. Oligoadenylates formed on a poly U_D or poly U_L template from DL-adenosine derivatives should, therefore, be optically homogeneous. Although we have not excluded the formation of L-chains on a D-template, we think this less likely than the formation of D-chains.

The work of Huang and Ts'O (*5*) shows that A_D and A_L form triple helices with poly U_D, which are of comparable stability. The specificity of the condensation reaction probably has its origin in the different relative orientations of 5′-phosphate and 2′-hydroxyl groups for D- D- and D-L- neighbors, but it could also be due to the segregation of enantiomers on the template.

Our results suggest that, when template-directed synthesis is predominant, optically homogeneous oligonucleotides can, in principle, be formed from initially racemic mixtures. If the formation of a "nucleus" for effective synthesis is highly improbable, then all templates in a given microenvironment would arise from a single ancestor and, in all probability, have the same configuration. Otherwise equal numbers of D- and L-chains would be expected. The important point for discussions of the origin of life is that optically homogeneous chains have a selective advantage whenever template synthesis occurs.

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3. Abbreviations: polyU$_D$, poly-D-uridylate; A$_D$, D-adenosine; A$_L$, L-adenosine; A, adenosine (optical isomer not specified); pA$_D$, D-adenosine-5′-phosphate; ImpA$_D$, D-adenosine-5′-phosphorimidazolide; NH$_2$pA$_D$, D-adenosine-5′-phosphoramidate; A$_L$pA$_D$, L-adenylyl-D-adenosine; optical density unit measured at 259 nm. See also reference (*1*).
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5 August 1968

Cigarette Smoke: Protection of Alveolar Macrophages by Glutathione and Cysteine

Abstract. *Phagocytosis of bacteria by rabbit alveolar macrophages is inhibited quantitatively by cigarette smoke in vitro. This phagocytoxic effect was abolished by addition of 0.2 to 0.4 micromole of glutathione or cysteine per milliliter of cigarette smoke. Serum protein was required to obtain both the toxic effect of the smoke and the protective action of the sulfhydryl compounds. The protective role of the sulfhydryl agents suggests an oxidant action of the cigarette smoke on these pulmonary cells.*

Among its many actions on lung function, cigarette smoke interferes with the phagocytic activity of alveolar macrophages. When freshly drawn cigarette smoke is introduced into a tissue culture flask containing alveolar macrophages and bacteria, the normally effective bactericidal action of these cells is markedly impaired (*1*). Microscopic examination of stained smears shows a marked reduction in the number of intracellular particles. The effect is quantitative and dose related. Six milliliters of cigarette smoke produced a maximal inhibitory effect. As a hypothetical comparison with human exposure, a comparable dose might be delivered to lungs of average capacity by a minimum of three cigarettes.

Various reducing agents were studied for their possible protective action for the cells against cigarette smoke. Alveolar macrophages were obtained by washout of rabbit lungs, centrifuged, and suspended in Hanks balanced salt solution containing 5 percent autologous rabbit serum. Approximately 10^6 cells were placed in 30-ml plastic tissue-culture flasks in a total volume of 2 ml, which contained also approximately 10^5 *Staphylococcus albus* bacteria and appropriately added chemicals. After all additions were made and the mixture sampled for quantitative bacterial culture, 6 ml of freshly drawn, whole or filtered cigarette smoke was introduced by syringe. The flasks were tightly stoppered, allowed to incubate for 2 hours at 37°C, and again sampled for quantitative bacterial cultures. The change in numbers of viable bacteria in each flask over the 2-hour period was taken as the index of macrophage function. Thus, the method does not distinguish between particle uptake and intracellular destruction.

The percentage of reduction of culturable bacteria seen in control flasks was reproducible within the experiment, although there was variability in the absolute numbers from experiment to experiment. For this reason, conclusions were based on comparison between control and treated flasks within each experiment.

Glutathione, added at the start of the experiment, inhibited the reduction in phagocytosis caused by cigarette smoke (Fig. 1). In control flasks 60 to 80 percent of the bacteria were killed in the 2-hour assay period. Similar phagocytic activity was noted in the flasks containing 2.5 micromoles of glutathione per milliliter, and no smoke.

Complete protection against the phagocytoxic action of the cigarette smoke was gained at a concentration of 2.5 micromoles of glutathione per milliliter of solution in the flask. There was no difference between control and smoked cells in the percent of bacteria killed. This protective effect was absent

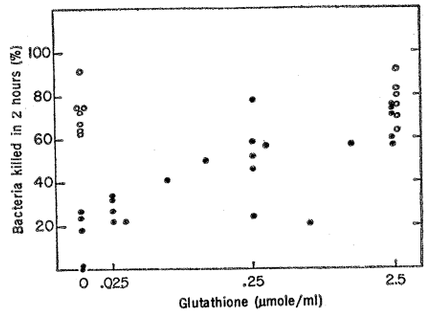

Fig. 1. Effect of glutathione on phagocytoxic activity of cigarette smoke. ○, No smoke added; ●, 6 ml of smoke added.

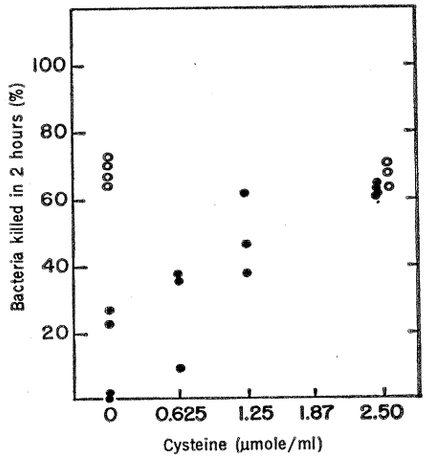

Fig. 2. Dose effect of cysteine on phagocytoxic action of 6 ml of cigarette smoke. ○, No smoke added; ●, 6 ml of smoke added.

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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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