placed only in those patches which bud out to form virus. The latter must be the case, since the specific activity of the isolated plasma membranes (H^3, 10,080 count/min per microgram; C^{14}, 1820 count/min per microgram) shows most of the original host cell protein is retained along with new protein synthesized at the direction of virus. Of course, this would vary according to time of labeling, but there is much more host protein in the plasma membrane than in the virus which buds off from it.

If it is assumed that the first viral-membrane proteins to reach the plasma membrane act as nucleation points for a further aggregation of more of the same molecules either at random sites on the membrane, or perhaps at certain "weak" points where there is little or no host protein, then localized patches of membrane in which the only protein associated with the lipid components is virus-directed would appear. After these patches grow to a critical size they tend to attract and engulf nucleocapsid cores, and to bud away from the cell as part of a virion; thus, a cell's plasma membrane could form countless virions by budding without losing all of its normal membrane proteins.

This gel electrophoresis technique is capable of resolving the "structural proteins" of the rather simple influenza virus membrane as discrete peaks. This is in marked contrast to the hundreds of proteins observed by the same technique in normal cell membranes, and it is further evidence against the presence of one or a few major structural proteins in normal cell membranes (*1*).

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Polyinosinic-Polycytidylic Acid Inhibits Chemically Induced Tumorigenesis in Mouse Skin

Abstract. *Polyinosinic-polycytidylic acid administered intraperitoneally inhibits (i) the formation of skin tumors induced by a single topical application of 9,10-dimethylbenzanthracene followed by weekly applications of croton oil and (ii) the formation of skin tumors induced by a single large application of 9,10-dimethylbenzanthracene.*

A single small dose of 9,10-dimethylbenzanthracene (DMBA) topically applied to mouse skin initiates tumorigenesis (*1*). The initiated foci may then be promoted to the visible tumor stage by the weekly application of a promoting agent such as croton oil (*1, 2*). Tumorigenesis may also be induced by a single large topical application of DMBA with no subsequent treatment with croton oil. The molecular mechanism of tumorigenesis is not known. The chemical carcinogen may directly transform normal cells to the tumor state, may activate latent tumor viruses, or may permit the development of either preexisting or carcinogen-transformed tumor clones which are normally suppressed (*3, 4*). The latter may relate to the role of immunological mechanisms in tumorigenesis. One theory of carcinogenesis suggests that tumor cell clones are normally inhibited from further development by immunological processes (*4*). A failure of normal immunological suppression would result in the development and appearance of gross tumors. The chemical carcinogen or promoter may, through immunosuppressive activity or by increasing the number of tumor cells formed, allow the formation of gross tumors. This may occur when normal immunological exclusion of tumor cells is overcome.

The synthetic double-stranded RNA, polyinosinic-polycytidylic acid (poly I • poly C) induces cells to synthesize interferon and to develop strong resistance to virus replication (*5*). In addition, synthetic polynucleotides enhance the immunological capability of the host as shown by an increase in circulating antibodies (*6*). Also, poly I • poly C inhibits the growth of several transplanted tumors (*7*). We now re-

Table 1. Effect of poly I • poly C on skin tumorigenesis at 11 to 12 weeks initiated by DMBA and promoted by croton oil. Each group consisted of 35 male Swiss mice weighing 18 to 22 g. The initial treatment was given in week 1; subsequent treatment was given after week 1. In experiment 1, 25 μg of DMBA in 0.2 ml of acetone was applied topically to the backs of cleanly shaven mice. Poly I • poly C (200 μg/0.2 ml per mouse) was given to group 3 on days 1, 2, 4, 6, and 9. The DMBA was given on day 2. In group 5 six doses of poly I • poly C (200 μg per 0.2 ml) was given on days 1, 3, 5, 7, 9, and 11. The DMBA was given on day 5. Mice in groups 5 and 6 received subsequent injections of 0.2 ml of poly I • poly C thrice weekly. All mice received topical applications of 0.2 ml of 1 percent croton oil once a week, starting 1 week after DMBA was given. All mice not given poly I • poly C received saline. In experiment 2, group 2 received five injections of 100 μg of poly I • poly C per 0.1 ml on days 1, 3, 4, 6, and 8. Group 4 received 100 μg of poly I • poly C per 0.1 ml thrice weekly, starting 1 week after DMBA was given. All mice received croton oil treatment as in experiment 1. All mice not given poly I • poly C received saline.

Group	Treatment		Survivors (No.)	Mice with tumors (No.)	Total tumors (No.)	Tumors per mouse (av. No.)	Percent of DMBA control
	Initial	Subsequent					
			Experiment 1A				
1	Acetone	None	31	0	0	0	0
2	DMBA	None	32	18	122	3.8	100
3	DMBA + Poly I • poly C	None	31	16	72	2.3	61
			Experiment 1B				
4	DMBA	None	30	17	102	3.4	100
5	DMBA + Poly I • poly C	Poly I • poly C	18	2	2	0.1	3
6	DMBA	Poly I • poly C	22	5	7	0.3	9
			Experiment 2A				
1	DMBA	None	30	28	154	5.1	100
2	DMBA + Poly I • poly C	None	28	25	166	5.9	116
			Experiment 2B				
3	DMBA	None	28	24	161	5.8	100
4	DMBA	Poly I • poly C	19	9	15	0.8	14

Table 2. Effect of poly I • poly C on the development of skin tumors induced by DMBA and promoted by croton oil. Groups are the same as in Table 1. Group 4 had 30 survivors after 20 weeks. Survivors in groups 5 and 6 given in parentheses. Group 4 received DMBA only; group 5 received DMBA + poly I • poly C (for 12.5 weeks); group 6 received DMBA + poly I • poly C (for 11 weeks).

Week	Number of tumors and survivors		
	Group 4	Group 5	Group 6
10	23	1 (24)	1 (24)
11	43	2 (22)	5 (23)
12	102	2 (18)	7 (22)
Treatment with poly I • poly C stopped			
13	109	12 (15)	14 (20)
14	161	31 (14)	17 (20)
15	198	50 (12)	22 (19)
16	199	57 (12)	29 (18)
17	318	88 (12)	89 (17)
18	319	104 (12)	81 (17)
19	326	122 (12)	92 (17)
20	330	125 (11)	95 (16)

port that poly I • poly C suppresses mouse skin tumorigenesis induced by DMBA alone or tumorigenesis initiated by DMBA and promoted by croton oil.

Groups of 30 male mice of the NIH general-purpose Swiss strain were treated as follows. The hair was removed from the back with electric clippers 1 day before the start of the experiment. The DMBA and subsequently the croton oil were applied topically to the entire back. The mice were examined weekly for visible papillomas. Skin tumors represent those tumors persisting for at least 2 weeks and measuring 1 mm or more in diameter.

Both poly I and poly C (P-L Biochemicals) were heterogeneous in size, with molecular weights in excess of 10^5. They were dissolved at a concentration of 1 mg/ml in pyrogen-free 0.85 percent NaCl at 44°C, neutralized to *p*H 7.7, and sterilized by filtration through a Millipore filter; the separate solutions of poly I and poly C were mixed under sterile conditions at equimolar concentrations. There was a hypochromic shift of about 50 percent, indicating that the base-paired double-stranded structure poly I • poly C had formed.

When the poly I • poly C was given intraperitoneally only shortly before and after the initiating DMBA treatment, there was only slight inhibition of tumor formation (Table 1, experiment 1A) or no inhibitory effect (Table 1, experiment 2A). In experiment 2A the dose of poly I • poly C was one-half of that given in experiment 1A. The significance and dose dependency of this possible inhibition is not known. When the poly I • poly C was given throughout the period of treatment with croton oil (Table 1, experiments 1B and 2B), there was suppression of tumor formation at 12 weeks. The suppression of tumorigenesis ranged from 86 to 98 percent. Treatment with poly I • poly C was stopped at 12 weeks because of toxicity which resulted in the death of some of the mice that were continuously treated with these polynucleotides. These mice, however, showed no weight loss and no enhancement of the skin toxicity to croton oil. In fact, the mice receiving poly I • poly C showed slightly less skin damage than the mice receiving only croton oil.

At 12 weeks treatment with poly I • poly C was discontinued, but all mice continued to receive croton oil (Table 2). Several weeks after treatment was stopped, large numbers of tumors began to appear. Thus, poly I • poly C did not reverse the state initiated by DMBA but rather prevented its promotion by croton oil. We do not know whether further treatment with poly I • poly C would continue to suppress tumorigenesis.

Table 3 shows that poly I • poly C effectively suppresses tumor formation induced by a single large dose of DMBA. This suppression by poly I • poly C was seen when poly I • poly C was given only for 2 weeks and no toxicity was observed. After 12 weeks the group treated with poly I • poly C exhibited only six tumors in five mice compared with 34 tumors in 15 mice in the group treated with DMBA only. The inhibition was observed up to 18 weeks after the initial treatment with DMBA, but this inhibition did not persist thereafter (Table 3). This indicates that poly I • poly C is also an effective suppressor of tumor formation when given simultaneously with the tumorigen. This is true when tumors develop after initiation by DMBA and promotion by croton oil as well as when a single large dose of DMBA is the sole agent inducing tumor formation.

We do not know the nature of the poly I • poly C suppression. An agent such as poly I • poly C which exhibits little toxicity to mouse skin and greatly inhibits skin tumorigenesis may represent a unique clue to the mechanism of normal physiological resistance toward chemical carcinogenesis. Several possible mechanisms can be considered for the poly I • poly C effect. The carcinogen or promoter may induce a viral activation or replication which may be inhibited by poly I • poly C-induced interferon formation. Polycyclic hydrocarbons inhibit interferon formation (*8*), and actinomycin D inhibits mouse skin tumorigenesis (*9*). The actinomycin D inhibition appears related to its inhibitory effect on DNA synthesis which seems required for tumor initiation (*9*). This requirement may be related to the fixing of an altered gene expression which may impart new antigenic character to the tumor cell. Tumors induced by chemical carcinogens exhibit new antigenic character (*10*), and this new antigenic behavior persists on further cell passage (*11*). Carcinogens also exhibit an immunosuppressive effect which interferes with the normal immunological defenses of the host (*12*). Poly I • poly C induces a high immunological capability as shown by its strong enhancement of graft versus host rejection mechanisms (*13*). This action of poly I • poly C may be an important part of its activity as a suppressor of chemically induced tumorigenesis. A number of reports indicate that immunological impairment may effect the course of chemical carcinogenesis. Thus neonatal thymectomy increases the incidence of lung adenomas

Table 3. Effect of poly I • poly C on the number of skin tumors in mice at 12 and at 22 weeks after a single large dose of DMBA. Each group consisted of 30 male Swiss mice. The initial treatment was the application of 250 μg of DMBA per 0.2 ml to the back of each mouse. Poly I • poly C (100 μg in 0.1 ml of saline) was given intraperitoneally thrice to each mouse in group 2 during the week that DMBA was applied. Mice not given poly I • poly C received saline. None of the mice received croton oil treatment.

Group	Treatment	Survivors (No.)	Mice with tumors (No.)	Total tumors (No.)	Tumors per mouse (av. No.)	Percent of DMBA control
	Effect after 12 weeks					
1	DMBA	29	15	34	1.2	100
2	DMBA + poly I • poly C	30	5	6	0.2	17
	Effect after 22 weeks					
1	DMBA	22	18	57	2.6	100
2	DMBA + poly I • poly C	25	21	52	2.1	81

induced by DMBA (*14*) and enhances skin tumor formation induced by benzo(*a*)pyrene (*15*). Under other conditions, however, an increased susceptibility to chemical carcinogenesis related to impaired immune states has not been confirmed (*16*). Poly I · poly C may be operating by counteracting the immunosuppressive effect of the carcinogen and may generally enhance the ability of the host to exclude antigenically different tumor cells. Poly I · poly C and other polynucleotides may be of use in enhancement of interferon production or immunological capability and may thereby represent a means of investigating the role of these processes in chemical carcinogenesis.

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Critical Flicker-Fusion of Solid and Annular Stimuli

Abstract. *Critical flicker-fusion thresholds were measured with both solid disks and annular stimuli. The former subtended 1, 4, and 8 degrees of visual angle, and the latter subtended 4 and 8 degrees. The illuminated region of the 4-degree annulus provided the same area of stimulation as that of the 1-degree disk. The illuminated region of the 8-degree annulus provided the same area of stimulation as the 4-degree disk. Annular stimuli significantly reduced critical flicker-fusion thresholds relative to those obtained with disk stimuli of the same area. The effects are not explicable in terms of heterogeneous retinal sensitivity. Laws relating critical flicker-fusion to area seem to be valid only under the restricted condition of homogeneous stimulation.*

Studies of the effects of various factors upon the critical flicker-fusion (CFF) threshold are among the most numerous in vision research (*1*). Thresholds are known to depend upon both the parameters of stimulation and certain characteristics of the organism, such as age, visual adaptation, and integrity of the visual pathways. Among the most reliable CFF relationships are those describing the effects of stimulus intensity, area, and position on the retina. The differential functioning of rods and cones is demonstrable through measurements of CFF thresholds over a range of luminances and positions from fovea to extreme retinal periphery (*2*, *3*). There is also spatial integration in that CFF thresholds are systematically elevated by increases in stimulus area (*4*). Theoretical statements have been summarized (*5*).

This study was prompted by a paradox that prevails in studies of the effects of stimulus area upon CFF. Since, with one known exception (*6*), all such investigations have employed solid circular stimuli, distinctions between effects due to stimulus size and those due to the retinal locus of stimulation have not been clear. The CFF threshold increases as the size of the stimulus is increased, but it decreases as stimuli are positioned further toward the periphery. The former effect is usually attributed either to spatial-summation effects or to the increased probability of stimulating the most sensitive retinal elements (*5*). The reduction in CFF which attends the displacement of stimuli toward the periphery is generally explained in terms of the reduced temporal resolving power of rods relative to that of cones (*3*).

In an attempt to distinguish between the effects of size and those of position, I employed both solid and annular stimuli. The diameters of the disk stimuli subtended 1, 4, and 8 degrees of visual angle while the outer diameters of the annuli subtended 4 and 8 degrees. The stimulating area of the 4-degree annular stimulus was the same as that of the 1-degree disk, and the stimulating area of the 8-degree annulus was the same as that of the 4-degree disk. Luminance was varied over 2.4 log units, the dimmest stimulus being a value that just allowed clear recognition of a constantly presented 1-degree disk after subjects had been adapted to darkness for 5 minutes. Annular stimuli were not visible at this intensity but were visible at a value 0.32 log higher. From the dimmest value (approximately 5 mlam), luminance was increased in log steps of 0.32, 1.0, 1.32, 1.64, and 2.08; that is, a range from 0 to 2.4 log units of intensity. To effect these increases, I used Wratten neutral-tint filters.

Stimuli were presented by a Scientific Prototype Three-Channel tachistoscope, modified to reduce the viewing distance to 14 inches (35 cm). Four subjects, restrained by a chin rest and a forehead support, viewed stimuli. Viewing was monocular and a dim red 0.5-degree patch was constantly present for foveal fixation. Stimuli were presented in 1-second bursts to further restrict fixation.

Thresholds were determined in ascending and descending series of measurements. In the former case, flicker rates were low enough to allow the flicker to be detected easily. Rates were increased from this value by a slow, continuous, and equal reduction in the duration of both the light and the dark components of the light-dark cycle. The light-dark ratio (LDR) on all trials was 1.0. A reverse procedure was employed in descending threshold determinations, beginning with repetition rates well above the fusion threshold and gradually reducing these rates until "flicker" was just detectable. For any given value of luminance and area of stimulus, the threshold was taken as the arithmetic average of two or three ascending and two or three descending threshold measurements. Whether two or three measurements were taken depended upon the variability of judgments to that point. From the beginning to the end of each 1-hour session, luminance was